Miltenyi Biotec

MACSQuant® Analyzer & MACSQuantify™ Software

User manual
Version 2

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Version 2.0

Miltenyi Biotec GmbH
Friedrich-Ebert-Straße 68
51429 Bergisch Gladbach
Germany
Phone: +49 2204 8306-0
Fax: +49 2204 85197
E-mail: macs@miltenyibiotec.de
www.miltenyibiotec.com

Software release 2.0.0945.R

Thank you for choosing a Miltenyi Biotec product.

The MACSQuant® Analyzer is an innovative instrument for automated multiparameter cell analysis.

Excite and inspire.

1 Important information

Please read before use!

Please read all information contained in this user manual before use. Failure to read and follow these guidelines could lead to improper or incorrect use, handling or care of your instrument and could cause hazards to users, unpredictable results, device malfunction or damage, premature wear and reduced life time of the instrument, and may void your warranty.

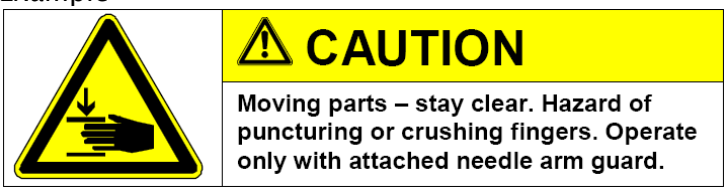
Keep this user manual in a safe place, accessible for anyone using the MACSQuant Analyzer.

This chapter describes the safety instructions and site requirements for your MACSQuant Analyzer. The following warnings and cautions are provided to help you prevent injury to yourself or damage to the device.

1.1 Symbols and hazard levels

1.1.1 Setup of safety notices

Example



The safety notices inform the user about potential risks if warnings and precautions outlined below are not followed. The icon on the left side specifies the risk. The hazard level at the top classifies the hazard, as mentioned above. The level, type, and source of the hazard as well as potential consequences, prohibitions, and measures are pointed as follows.

1.1.2 Symbols

The following chart is an illustrated glossary depicting the symbols that are used in this user manual and on the MACSQuant Analyzer.

⚠ CAUTION	Indicates a hazard situation, which if not avoided, could result in minor or moderate injury.
⚠ WARNING	Indicates a hazardous situation which, if not avoided, could result in death or serious injury.
	Attention, consult the User Manual for further instructions and proceed with caution. Warnings include the risk of damage to the equipment, severe personal injury, or loss of life
	Hazard of crushing and shearing. Risk of crushing and shearing of bodily parts due to mechanical hazards.
	Laser radiation Risk of serious eye and skin injuries.
	Strong permanent magnet Contains a strong permanent magnet. Magnetic devices can interfere with electronic devices or damage magnetic information carriers.
	Risk of contamination if biohazardous material is used. Indicates the risk of loss of life, severe injury to the instrument operator, or equipment damage due to potentially dangerous biological material.
	Indicates the risk of loss of life or severe injury to the instrument operator due to hazardous voltage.
	Protective conductor terminal Symbol is attached on the inside of the instrument. Warning for service personnel.
	On (supply)
	Off (supply)

1.2 Warnings and precautions

The MACSQuant Analyzer is a novel, computer-controlled device for flow cytometry. Cells isolated using renowned MACS Technology may be subsequently measured using the MACSQuant Analyzer. The MACS MiniSampler connects to the MACSQuant Analyzer and thus represents a part of the instrument. The MACSQuant Analyzer and the MACS MiniSampler are designed to operate safely after installation and use by trained personnel according to general safety practices and the instructions set forth in this user manual. The guidelines in this section explain the potential risks associated with the operation of the instrument and provide important safety information in order to

minimize these risks. By carefully following the instructions, you can protect yourself and the equipment from potential hazards and create a safe work environment. If this instrument is used in a manner not specified by the manufacturer, user safety may be compromised.

IMPORTANT: Please read and follow all operating instructions in this user manual and pay attention to all warnings displayed on the instrument. Retain this user manual and any other safety and operating instructions provided with the instrument in a place accessible to all users for future reference.

IMPORTANT: The MACSQuant Analyzer is intended for indoor use only. Do not use the instrument in areas classified as hazardous locations such as oxygen-laden environments.

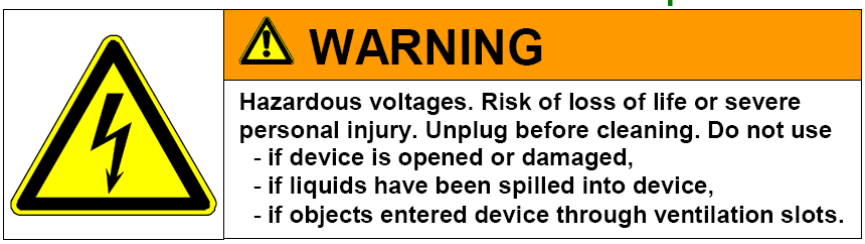
Contact your local authority governing electrical power supply, building constructions, maintenance, or safety for more information regarding the installation of the equipment.

If you have a serious concern regarding the safe use of your instrument, please contact your authorized Miltenyi Biotec service provider or call Miltenyi Biotec Customer Service.

1.3 General precautions

To reduce potential risks associated with operating the MACSQuant Analyzer, please observe the following general precautions. Failure to observe these precautions could result in fire, bodily harm, and/or damage to the instrument.

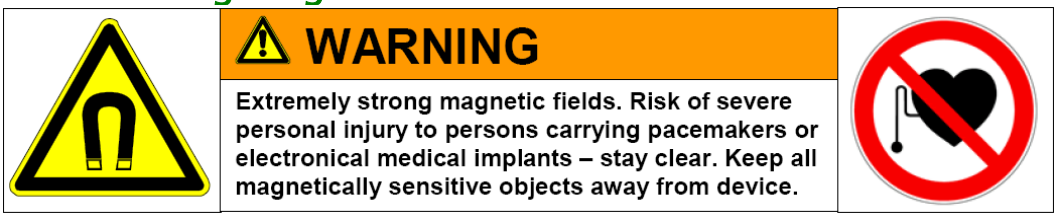
1.3.1 Hazard of electric shock and spread of fire



Warning: Electrical devices pose the risk of an electric shock. To reduce the risk of an electric shock, do not open any cover other than the front access covers of the MACSQuant Analyzer nor any other accessory hardware supplied by Miltenyi Biotec. All other covers of the device and accessory hardware are to be removed by authorized personnel only. Special care must be taken while handling fluids. Clean up spillages immediately. Do not allow fluids to enter the interior of the device. Unplug the power cord before manually cleaning the MACSQuant Analyzer.

Warning: A potential risk exists if an opened, dropped or damaged MACSQuant Analyzer is used, if liquids are spilled into the instrument, if an object has entered the instrument through the ventilation slots, or if an object has been dropped into the instrument. If flames or smoke appear immediately switch off the MACSQuant Analyzer, unplug the instrument from the electrical outlet, and contact an authorized Miltenyi Biotec service provider or the Miltenyi Biotec Customer Support team. Use of a damaged instrument or an instrument with a damaged power cable is expressly prohibited.

1.3.2 Strong magnetic field



Warning: The MACSQuant Analyzer is equipped with an extremely powerful magnet. Keep any magnetic information carriers (such as credit cards, magnetic tapes and floppy disks) and any electronic equipment (such as hearing aids, pacemakers, measuring and control instruments, computers, and watches) at a distance of at least 20 cm from the magnet cover. These items may be affected or damaged by the magnetic field.

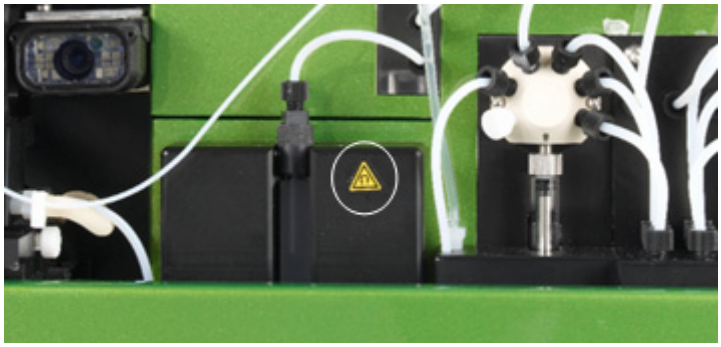
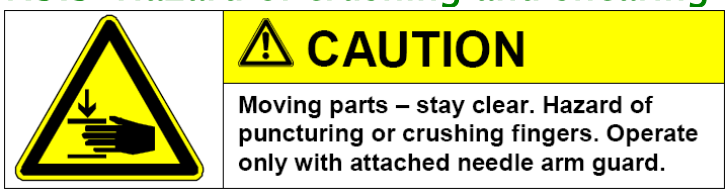


Figure 1.1 Location of warning sign for strong permanent magnet.

1.3.3 Hazard of crushing and shearing



CAUTION: Do not open the front access covers while the device is in operation. Do not obstruct the movement of the automated arm and accessory hardware during operation. Keep fingers etc. away from all moving parts of the MACSQuant Analyzer and accessory hardware, to avoid crushing or shearing injuries, or damage to the

device. Do not touch fluid pumps or adjust the tubing, while the device is in operation. Always switch off the device before adjusting any part of the fluidic system. Always stop or abort a procedure before handling accessory hardware, e.g. MACS MiniSampler, or loading/removing tubes from the tube rack placed on the sampler. Do not circumvent any safety measures or devices.

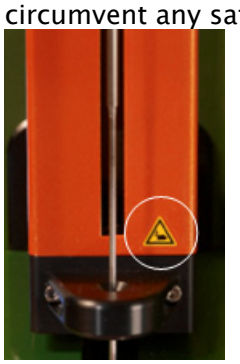


Figure 1.2 Open circle shows warning sign for 'hazard of crushing and shearing'.

1.3.4 Laser and LED radiation

The MACSQuant Analyzer is equipped with continuous-wave lasers, vertical-cavity surface-emitting lasers (VCSEL) and powerful light emitting diodes (LED).

Warning: The device is equipped with up to three continuous-wave lasers (class 3B laser) for fluorochrome excitation. These lasers are secured by protective housing. Do not remove the protective housing. Otherwise, eye injury may result.

The device is also equipped with four VCSELs for automated rack detection (class 1M laser). The laser radiation from these devices is not visible. Do not view directly with optical instruments (e.g. lenses, magnifying glasses, or microscopes). Viewing the VCSEL port within a 100 mm distance using optical instruments could be hazardous to the eye.

Do not intentionally direct the laser beam at others.

The device is also equipped with powerful LEDs for the illumination of the supply bottles and with a 2D Code Reader which uses powerful LEDs for illuminating the reading area (class 1 LED). Do not look directly at LED radiation or reflected LED radiation from a mirrored surface. Otherwise, eye injury may result. Do not disassemble, modify or remove the installed laser radiation sources or their mounting rackets. The laser radiation sources do not automatically stop emitting when disassembled.

Do not allow water, oil, dust, or other foreign substances to stick to 2D Code Reader aperture window. This may cause read errors. Use a soft, dry cloth to wipe any substances from the scanner. Do not use alcohol or other cleaning substance.

Radiation of disassembled units may lead to eye injuries.

The MACSQuant Analyzer is classified as a Class 1M laser product per standard IEC 60825-1: 1993 + A1: 1997 + A2: 2001.

CAUTION: Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.

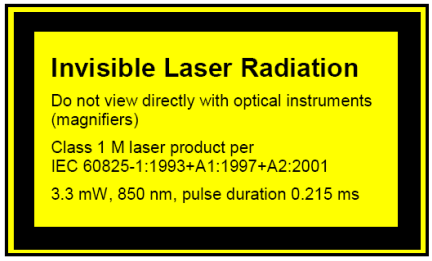


Figure 1.3 Position of lasers and LEDs. Invisible rack detection lasers (VCSELs) are located within the rectangle area. The 2D code reader (visible) is located within the open circle.

1.4 Secure installation

This section describes the requirements your site must meet for safe installation and operation of your MACSQuant Analyzer. Read the instructions in this section and ensure that your site is properly prepared before you connect the instrument to its power source.

When planning your site layout and equipment locations, keep in mind the precautions described in this section to help avoid instrument failures and reduce the possibility of environmentally caused shutdowns.

IMPORTANT: At all times, local working area safety instructions, laboratory policies, and standards regarding laboratory health and safety and prevention of accidents must be adhered to.

1.4.1 Mounting accessories

Do not place the MACSQuant Analyzer on an unstable table, cart, stand, tripod, or bracket. As a consequence, the instrument might fall down. This may cause serious bodily harm and/or serious damage to the instrument. Use only on a table, cart, stand, tripod, or bracket that can easily support a weight of 50 kg. Do not place the MACSQuant Analyzer within a built-in apparatus or a confined space such as a shelf rack unless the apparatus has been specifically designed to accommodate the instrument, proper ventilation is provided, and the mounting instructions for the instrument have been followed.

1.4.2 Air circulation

Ambient air temperature might not be adequate to cool the MACSQuant Analyzer to acceptable operating temperatures without adequate circulation. Make sure that the room in which you operate the instrument has adequate air circulation. The instrument

should not be placed next to radiators, heat registers, stoves, or other pieces of equipment (including amplifiers) that produce heat. Allow sufficient air circulation around the MACSQuant Analyzer—at least 15 cm on all sides—during operation to ensure adequate cooling of the instrument. Prevent direct exposure of the instrument to sunlight. Slots and openings of the instrument are provided for ventilation and should never be blocked or covered, as these ensure reliable operation of the MACSQuant Analyzer and protect the device from overheating. Never push a foreign object through an opening into the instrument.

1.4.3 Water and moisture

Do not use the instrument in a wet or damp location. Avoid high humidity or condensation and protect the machine against water splashes.

1.4.4 Grounded (earthed) product

The instrument is equipped with a three-wire electrical grounding-type plug that has a third pin for grounding. This plug only fits into a grounded power outlet. This is a safety feature. Do not try to insert the plug into a non-grounded power outlet. If you cannot insert the plug into the outlet, contact your local electrician to replace the outlet.

1.4.5 Power sources

The instrument should only be operated from a power source indicated on the product's electrical ratings label. If you have questions about the type of power source to use, contact your authorized Miltenyi Biotec service provider or local power company. Do not use extension cords or power strips. Do not overload an electrical outlet. The overall system load must not exceed 80% of the branch circuit rating.

1.4.6 Accessibility

Make sure that the main switch as well as the connector for the power cable are easily accessible and located as close to the operator of the instrument as possible. If it is necessary to disconnect the power supply, unplug the cable from the power outlet.

1.4.7 Peripheral devices

Only original MACSQuant Analyzer Equipment shall be attached to the connectors labeled "External CAN", "CAN1", and "CAN2". The voltage levels on these connectors shall not exceed hazardous voltage levels of 30 Vrms. and 42.4 Vpeak or 60 Vdc. Only the MACSQuant Analyzer Bottle Sensor Cable should be attached to the "Bottle Sensor" connector. Only a 2D code reader recommended by Miltenyi Biotec should be connected to the "RS232/BCR" connector. External laser devices connected to the connector labeled "RS232/BCR" have to comply with the standard IEC 60825-1. External computing devices connected to the R232 interface connectors labeled "COM 1" have to be listed in accordance to the standard UL 60950-1. Only use connector cables less than 3 m in length.

1.5 Secure operation, maintenance, transport and disposal

Observe the following instructions to ensure secure operation, maintenance, transport, and disposal of your MACSQuant Analyzer.

IMPORTANT: At all times, local working area safety instructions, laboratory policies, and standards regarding laboratory health and safety and prevention of accidents must be adhered to.

1.5.1 Secure operation

If the instrument is not working properly and instructions or messages on the display screen advise to contact technical service, secure operation is no longer possible. Immediately switch off the MACSQuant Analyzer, unplug the instrument from the electrical outlet, and contact an authorized Miltenyi Biotec service provider or the Miltenyi Biotec Customer Support team.

1.5.2 Servicing

IMPORTANT: Unless otherwise specifically noted in this User Manual or other Miltenyi Biotec documentation, do not service the MACSQuant Analyzer yourself. Servicing and repair must be performed by qualified service personnel. Improper or incorrect servicing or repair of your MACSQuant Analyzer can cause hazards to users, lead to unpredictable results, device malfunction or damage, premature wear and reduced life time of the instrument, and may void your warranty.

Inquire with your local Miltenyi Biotec representative about Miltenyi Biotec's extensive instrument service and support arrangements, or see www.miltenyibiotec.com/support.

IMPORTANT: When replacement or spare parts are required, make sure that the service provider uses only genuine Miltenyi Biotec parts or third-party parts specified and recommended by Miltenyi Biotec. Using unauthorized replacement or spare parts can cause malfunction of the device and impair flow cytometry results. Miltenyi Biotec does not honor any warranty or accept any responsibility for device failure or damages resulting from the use of inappropriate replacement or spare parts. After completing any service or repair work, have your authorized Miltenyi Biotec service provider perform all safety checks required by the repair procedure to ensure that the instrument is in proper operational condition.

Only use options and upgrades recommended by Miltenyi Biotec.

1.5.3 External cleaning

Unplug the MACSQuant Analyzer from the outlet before cleaning. Do not use liquid or aerosol cleaning agents; always use a damp cloth.

1.5.4 Hazardous material

⚠ WARNING

Risk of loss of life if biohazardous material is used. Wear protective gloves, protective clothing, and safety glasses to prevent contact with skin and eyes. Operate device in a safety hood. Decontaminate device after spilling of biohazardous material.

If biohazardous material is or has been used, the operator shall choose and wear personal safety equipment in accordance with warnings and precautions for the used substances. Wear protective gloves, protective clothing, and safety glasses to prevent contact with skin and eyes. Also protect mouth and nose as aerosols might leak from the system (e.g. Washing Station). Defective or inadequate safety equipment might endanger the operator. The MACSQuant Analyzer shall be operated in a safety hood if hazardous or unknown materials are processed. If hazardous material has been used or spilled, care must been taken to thoroughly decontaminate the system. For details, see section 9.1.9.

Always inspect the fluidics system (complete tubing set, reservoirs, bottles and their closures, valves, columns, diluters, peristaltic pumps and needle) before switching on the device. If leakage has been detected, replace all damaged parts before switching on the device. If damaged parts cannot be replaced, unplug and do not use the device. Failure of parts containing biohazardous material or liquids that have been in contact with such material could cause a hazard.

Columns, tubes, and any other consumables that were in contact with biohazardous samples shall be autoclaved prior to disposal. Liquid waste shall be autoclaved or decontaminated using a disinfectant that is appropriate for the specific pathogen, e.g. 10% bleach, isopropyl alcohol, or 70% ethanol. Miltenyi Biotec recommend use of MACS Bleach.

Waste disposal must be in accordance with any local regulations.

Figure 1.4 Warning signs for biohazard on rear panel and waste bottle.

Safety check

After completing any service or repair work, have your authorized Miltenyi Biotec service provider perform all safety checks required by the repair procedure to ensure that the instrument is in a proper operational condition.

1.5.5 Transport

The MACSQuant Analyzer should be transported with care in packaging specified by Miltenyi Biotec. Internal damage can occur, if it is subjected to excessive vibration or if it is dropped. If the instrument needs to be shipped back to the manufacturer for service, decontaminate the instrument from any hazardous material prior to shipment. If you have questions regarding proper decontamination or shipment, please contact technical service for assistance. See section 9.1.9 for further information on instrument decontamination.

1.5.6 Instrument disposal

Please contact technical service for assistance if you wish to dispose of your instrument.

1.5.7 Electromagnetic compatibility

Changes or modifications of the equipment unless expressly approved by Miltenyi Biotec may void your authority to operate the equipment pursuant to 47 CFR §15.

NOTE: This equipment has been tested and found to comply with the limits for a Class B digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference in a residential installation. This equipment generates, uses and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

- Reorient or relocate the receiving antenna.
- Increase the separation between the equipment and receiver.
- Connect the equipment into an outlet on a circuit different from that to which the receiver is connected.
- Consult the dealer or an experienced radio/TV technician for help.

2 Introduction

2.1 Purpose

The MACSQuant® Analyzer is a benchtop flow cytometer that has been specifically designed for the rapid, simple, and automated fluorescence analysis of single-cell suspensions. The MACSQuant Analyzer also facilitates the absolute quantitation of cell populations and has a processing rate of up to 10,000 events per second. The instrument was designed for use with MACS® Cell Analysis and MACS® Separation Reagents in research applications, though common fluorochrome-conjugated antibodies from other suppliers can also be used.

The relatively small footprint of the MACSQuant Analyzer (60×35×40 cm) in comparison to other commercially available flow cytometers makes the instrument ideally suited to benchtop operation within the laboratory. Also, the instrument has several design features that permit the fully automated processing of cell samples—from sample labeling and mixing, through uptake, and magnetic enrichment, to fluorescence analysis.

The MACSQuant Analyzer can be optionally fitted with the MACS® MiniSampler. The MiniSampler is a motorized sample rack holder that can hold tube racks of varying formats including 96-well microtiter plates. The fully automated uptake and processing of multiple samples is possible under control of the MACSQuantify™ Software—thus permitting the user a hands-free, high-throughput operation.

Automated maintenance procedures are also a design feature of the MACSQuant Analyzer. This includes different system wash programs before each measurement, automatic priming of the instrument, and programs for shutting down the instrument for overnight or long-term storage.

After sample uptake, the instrument can analyze fluorescence labeled cells using up to nine optical parameters—seven fluorescence and two scatter channels. The software provided with the MACSQuant® Analyzer can perform standard flow cytometric data analyses and illustrations, including histograms, dot plots, density plots, gating, and statistical views. Data can also be collected in terms of time, area, height, and width. During acquisition, the data are automatically stored in user designated folders for later analysis. These folders are assigned for each user as either private or public access by the MACSQuant Analyzer administrator.

The MACSQuant® Analyzer can also optionally perform pre-enrichment of magnetically labeled cells before flow cytometric analysis. This feature is based on the renowned

MACS Technology and is particularly useful for the analysis of rare cells labeled with MACS MicroBeads.

Operation of the instrument is extremely simple through the use of the TFT color touch screen and intuitive screen menus built into the MACSQuant Software. The user has the option of performing simple analyses pre-programmed into the software using the **Express** mode or of customizing sample analysis protocols and automation programs using the **Custom** mode. Data analysis using a variety of display options and functions can be performed on the instrument and the software has been configured for user-friendliness and to provide highly flexible functionality.

Finally, standard MACSQuant® Analyzer Buffers and Solutions,, which are directly attached to the instrument, are sterile, ready-to-use and designed for optimal instrument performance. The instrument also provides a color-coded LED warning system which illuminates the fluid containers to inform the user when buffers need to be exchanged or waste removed.

2.2 Applications

The MACSQuant® Analyzer is more than just a flow cytometer. The instrument was designed with automation in mind and for use with MACS® Control reagents—fluorescence antibody conjugates supplied in an optimized format for the rapid flow cytometric control of cell separations performed with MACS Technology. The automated uptake of samples using the needle arm permits measurement of a pre-defined sample volume, which in turn permits an absolute quantitation of cells in a sample. According to their fluorescence labeling, different cell populations can therefore also be quantified. A brief summary of the main design features of the MACSQuant Analyzer is given below.

2.2.1 Fluorescence cell analysis

First and foremost, the MACSQuant® Analyzer is a flow cytometer comprising of nine optical channels for the measurement of fluorescence signals and the relative size and relative granularity of cells. In conjunction with the MACS® MiniSampler, the automated analysis of multiple samples can be performed with ease. The MACSQuantify™ Software performs all common functions for the presentation and statistical analysis of collected data. Data can be presented as dot plots, density plots, histograms, or statistical tables.

2.2.2 MACS® Control Applications

The quality of cell separations using MACS® Technology can be easily and rapidly assessed by the MACSQuant® Analyzer . Customized measurement and analysis protocols can be created for the fluorescence analysis of cells, while antibody labeling, processing, and sampling of cells can be performed in an automated fashion under the control of the MACSQuantify™ Software. Specialized MACS Control antibody cocktails

are also available for the multi-parameter analysis of certain cell types, including CD14+ monocytes and CD19+ B cells.

2.2.3 Rare cell detection

The inclusion of the MACS® Enrichment Unit within the system permits the magnetic enrichment of cells *in situ* prior to fluorescence analysis. The MACS® Enrichment Unit and the MACSQuant Column allow the possibility to reduce the number of cells analyzed to characterize the rare cell population of interest. This is particularly useful for the analysis of cells present in low abundance, such as stem cells, dendritic cell subsets, or NK cell subsets.

2.2.4 Absolute cell quantitation

The MACSQuant® Analyzer employs a robotic needle arm to acquire cell samples and to apply the sample into the flow cell. The robotic arm provides the advantage of automation and the ability to sample a specific volume. The MACSQuant Analyzer can therefore count an absolute number of cells per µL volume of sample (error margin ±5%). This also means that multiple cell populations can be simultaneously enumerated within a sample after fluorescence staining and analysis. For example, the ability of the MACSQuant Analyzer to provide absolute quantitation of cell populations permits the optimized enumeration of specific cell types using pre-defined software analysis protocols and specialized antibody kits. For example, using the CD4+ RTE Enumeration Kit (# 130-092-055), the enumeration of CD4+ recent thymic emigrant (RTE) cells is possible from whole blood samples or peripheral blood mononuclear cells (PBMCs). Furthermore, the automated processing of cells and the use of the MACS Mini Sampler facilitate the seamless incorporation of the MACSQuant Analyzer into routine, high-throughput laboratory cell enumeration procedures. Labeling reagents and software analysis can be customized to suit individual applications.

2.2.5 Automated cell labeling and analysis

Automation of cell sampling and analysis can be extended to include the labeling of cells with the MACSQuant Analyzer. With the use of the optional MACS® MiniSampler, the automated processing of up to 96 samples is possible facilitating the integration of the instrument into high-throughput procedures.

2.2.6 Flow cytometry—an introduction

Any given cell population can be defined by its individual expression profile of both intracellular and extracellular antigens. These antigens can therefore be targeted for their detection and further analysis using flow cytometry.

Flow cytometers detect cells according to two basic parameters: light scatter and fluorescence. Cell size and granularity are inherent characteristics of a cell and vary from one cell type to another; these properties are measured using the forward scatter (FSC) and side scatter (SSC) channels, respectively.

However, with the exception of transient or stable expression of fluorescence proteins, relatively 'bright' fluorescence that occurs above background auto-fluorescence requires cells to be stained with fluorescence dyes. This is normally achieved through the use of antibodies that target a specific protein or other biochemical antigens expressed on or within cells. These antibodies are either directly conjugated to a fluorochrome or can themselves be stained in a secondary step by a fluorochrome-conjugated secondary antibody (indirect staining). Only cells expressing the particular target antigen will be fluorescence-labeled.

After excitation by a laser, light emitted from fluorescence dyes can be detected in defined wavelength ranges. This differs from one fluorochrome to another. The use of different light filters in the flow cytometer permits the simultaneous use of multiple fluorescence dyes and thus the detection of multiple cellular antigens. These filters create fluorescence channels, which is monitored by a photomultiplier tube (PMT). Each PMT, which is located after a filter set, will amplify the signal of the detected light. Therefore, the laser will excite a fluorescence marker on the cell, which will be deflected by the cell and collected at a 90° angle. This deflected light will pass through the appropriate filter and the resultant signal will be amplified and reported by the flow cytometer software (i.e. the MACSQuantify Software). It is strongly recommended to assign meaningful names in the software for each photomultipler tube detector before beginning an analysis i.e. the naming nomenclature must correspond to the fluorochromes used for cell staining. For example, FITC (fluorescein isothiocyanate) has an emission maximum of 521 nm (in water) and is thus detected in the green fluorescence channel (FL2, 525 nm with a bandwidth of 50 nm). In contrast, APC (allophycocyanin), with an emission maximum of 660 nm, is measured in the red fluorescence channel (FL6, 655-730 nm).

The MACSQuant Analyzer is equipped with three lasers for measurement of up to seven fluorescence channels (FL1-FL7) and two scatter channels (FSC, SSC). For a list of representative fluorochromes and their respective detection channels see Table 2.1.

Excitation wavelength	Photomultipler tube (PMT) name	Filter
405 nm	FL1 (VioBlue)	450/50
488 nm	FL2 (FITC)	525/50
	FL3 (PE)	585/40
	FL4 (PE-Cy5/PE-Cy5.5)	655-730 (655LP + split 730)
	FL5 (PE-Cy7)	750 (LP)
635 nm	FL6 (APC)	655-730 (655LP + split 730)
	FL7 (APC-Cy7)	750 (LP)
488 nm	FSC/SSC	488/10

Table 2.1 Summary of compatible fluorochromes and respective channels.

After antibody labeling, the needle arm of the MACSQuant Analyzer draws a pre-definable volume of the cell sample into the instrument. Cells are either transferred directly to the flow cell for analysis or can be diverted to the MACSQuant Column for pre-enrichment of magnetically labeled cells (see section 6.3.6). Once in the flow cell, each cell individually passes through the path of a laser and the deflection of light from the cell is used to provide information on physical characteristics, such as size and granularity. Also, the laser light excites the fluorochromes on fluorescencely-labeled cells; the light emitted from each excited fluorochrome is measured by color detectors after passing through the respective filters (fluorescence channels). Finally, the cells are discarded into the waste container.

2.2.7 Displaying flow cytometric data

Flow cytometry data can be displayed in five different formats by the MACSQuantify Software: dot plot, histogram, density plot and statistic. Each category is briefly discussed below; however, it is worth noting that data are normally visualized as one-parameter histograms or two-parameter dot plots. For more information on using the MACSQuantify Software for data analysis and displaying charts refer to section 6.12.

Dot plot

A dot plot may also be referred to as “bivariant display”, “scattergram” or in some cases “bitmap”. In this form of analysis each cell event is represented as a single dot on a two-axis scale chart. The position of the dot on the x/y scale is dependent on the intensities of the measured parameters for that cell/event. Characterization of a cell population is typically achieved by displaying a dot plot where side-scatter (SSC; y-axis) is plotted against forward-scatter (FSC; x-axis).

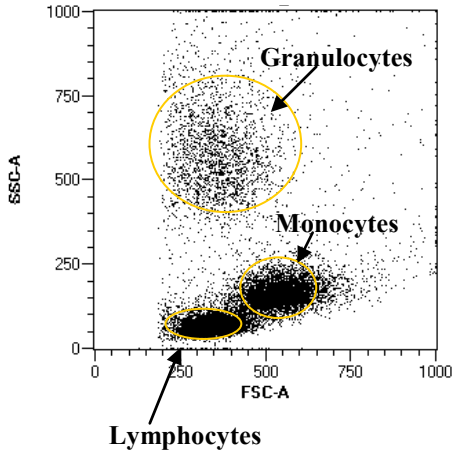


Figure 2.1 A two-parameter dot plot showing side-scatter plotted against forward-scatter of human peripheral blood mononuclear cells (PBMCs). Three distinct cells populations can be identified according to their light-scattering properties: granulocytes, monocytes and lymphocytes.

Scatter scales are usually plotted using a linear scale. In fluorescence dot plots the x- and y- scales are used to plot fluorescence intensity, and since fluorescence intensity can vary by several orders of magnitude between cells, a logarithmic scale is usually employed (e.g. fluorescence intensity spanning five decades; for more information about choosing an appropriate scale refer the section “Scaling flow cytometry data”

below). Dot plots are ideal for displaying relatively small numbers of events where discrete cell populations can be easily identified (e.g. see Figure 2.1**).** They also provide some indication regarding the relative density of cell events, i.e., with more events the dots accumulate forming a darker dot plot with more contrast. However, for a more accurate reflection of the relative density of cell events a **density plot** should be employed.

Histogram

Histograms are used to plot the intensity of a single parameter (x-axis) against the frequency of that parameter (y-axis); i.e., the x-axis represents scatter or fluorescence intensity and the y-axis axis represents the number of events. Since histogram charts can display only one dimension they should be employed for well resolved homogeneous populations or for comparing intensities of multiple samples for a single parameter (overlays).

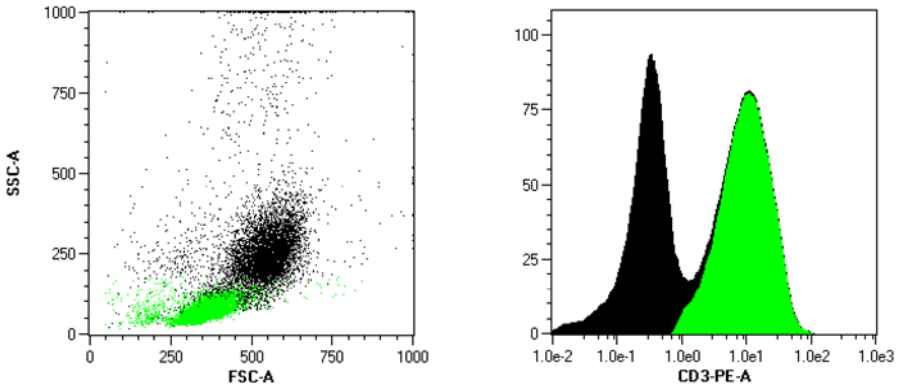


Figure 2.2 Left: FSC/SSC dot plot of PBMCs that were stained with CD3 antibodies conjugated to PE; CD3+ cells are depicted green. Right: Corresponding histogram of the CD3+cell population; fluorescence intensity (x-axis) is plotted against relative cell number (y-axis).

Density plot

Density plots are a useful tool. With traditional dot plots it may be difficult to quickly determine the intensity or frequency of acquired events on a black and white graphic. A density plot is plotted in grayscale or in color; each color/shade provides information about the intensity of acquired events. In essence, the density plot is designed to represent a three-dimensional plot, where the number of cell events are depicted in a 'third' dimension, either by shades of grey, or by using different colors.

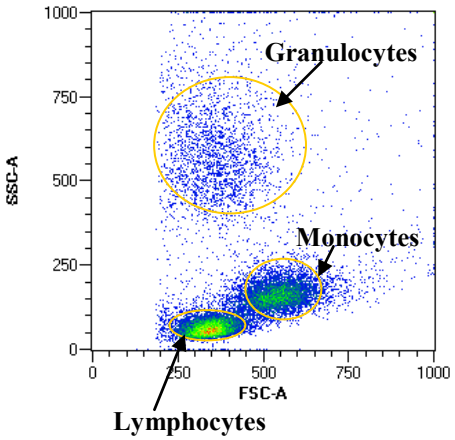


Figure 2.3 Representative density plot of peripheral blood mononuclear cells. The red color represents the highest density of cells, followed by yellow, then green and finally royal blue, which represents the cells occurring at the lowest frequency.

Statistics

Before performing statistical analysis of cell populations it is important to note that the precision and relevance of the analyzed data is dependent on the sample size and choice of statistic, respectively. The MACSQuantify™ Software can display a summary table of statistical attributes, namely: count, percentages arithmetic mean, coefficient of variation (CV), minimum/maximum/medium, median and modal statistics. In addition, since the MACSQuant Analyzer performs volumetric cell enumeration the actual cell count can also be displayed with each measurement.

Text

The MACSQuantify Software "Text" option is a text box that may be used to enter alphanumeric characters that may be used, for example, to document details about the experiment, the gating strategy or specific dot plot.

Scaling flow cytometry data using MACSQuantify Software

Standard dot plots showing side scatter and forward scatter typically use linear scales. This is often not possible when fluorescence labeled and non-fluorescence labeled cell populations are being analyzed as the difference in fluorescence signal intensities can extend over several orders of magnitude. As a consequence a logarithmic scale must be used. The impact of selecting an appropriate scale is exemplified by Figure 2.4 below. In this example a sample was analyzed which contained a population of white blood cells labeled with the fluorochrome phycoerythrin (PE). A linear scale was used to display a dot plot of forward scatter vs. side scatter (A) and to display a histogram of PE fluorescence intensity versus cell count (B). In Figure 2.4: B, the signal intensities of non-fluorescence cells and fluorescence labeled cells are "squeezed together". To separate the signals a log5 scale was used (Figure 2.4: C) revealing two peaks: the left peak is attributable to background fluorescence whereas the right peak is due to cells labeled with PE. As the MACSQuant Analyzer acquires data in a digital format some fluorescence intensities may be assigned a value less than zero. Data values less than zero may not be displayed properly using a conventional logarithmic scale, although

however, all calculated statistics will be correct. This is a general feature of more advanced “digital flow cytometers”. To overcome this, a hyperlog (Hlog) or biexponential scale may be used (D). In a Hlog scale the upper values of the scale are logarithmic whereas the lower values are linear.

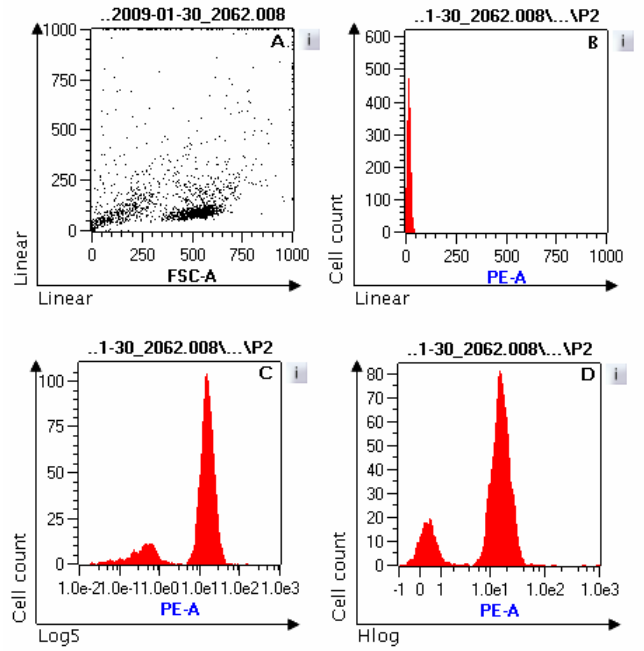


Figure 2.4 Comparing log and linear scales when displaying fluorescence and non-fluorescence events.
A: Dot plot showing forward scatter vs. side scatter using linear scales.
B: Histogram showing fluorescence intensity (linear scale) vs. cell count.
C: Histogram showing fluorescence intensity (log5 scale) vs. cell count.
D: Histogram showing fluorescence intensity (hlog scale) vs. cell count.

There are occasions when the difference between fluorescence values in a dataset are relatively small, for example, when using fluorescent probes to measure quantitative changes in cellular DNA during cell cycle. A linear scale must therefore be used in order to visualize these subtle changes (see Figure 2.5).

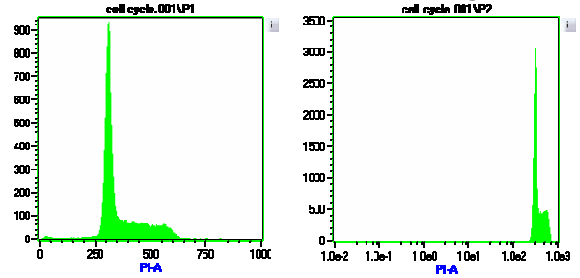


Figure 2.5 Measuring quantitative changes to cellular DNA during cell cycle. DNA was labeled using the fluorescent probe propidum iodide (PI). A linear scale (left) and log5 scale (right) was used to plot fluorescence intensity against cell count. Subtle changes to the quantity of DNA can only be visualized using a linear scale (left).

As a general rule, however, changes in fluorescence intensity usually span over several orders of magnitude and therefore a logarithmic or biexponential (hLog) scale should be used.

2.2.8 Analyzing flow cytometric data using regions or "gating"

It is usually necessary to analyze cell subpopulations or at the very least, to remove dead cells and debris from a dataset. This can be achieved by defining regions of interest or "gates" around certain cell populations. These "gates" are defined using geometric shapes and can be included or excluded in subsequent data analyses. The following geometric shapes can be used by the MACSQuantify Software to define regions.

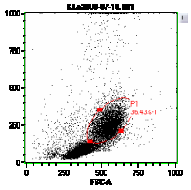
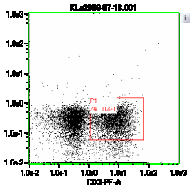
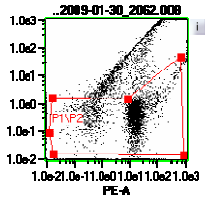
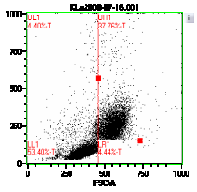
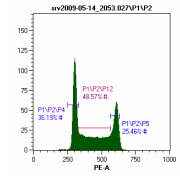
MACSQuantify Software icon	Name	Example
	Ellipse	
	Rectangle:	
	Polygon: Freehand shapes can be drawn.	
	Quadrant: Two-parameter dot plots can be subdivided into four quadrants. UL=Upper left, LL=Lower left; LR=Lower right; UR=Upper right.	
	Intervals or markers can be drawn on histograms to calculate statistics for designated regions.	

Table 2.2 Geometric shapes that can be used to draw "gates" or regions of interest using the MACSQuantify Software.

Complex or boolean gating

Several regions can be defined to form a boolean argument or a "gating strategy" which only displays highly specific cell populations; for example, cell populations with a defined set of scatter properties and/or a specific cell surface marker phenotype as

identified by fluorescence labeling using fluorochrome-conjugated monoclonal antibodies.

Gates or regions created using MACSQuantify Software are identified by the letter “P”, where “P” is derived from “Population”. An example of a complex gating strategy is shown by Figure 2.6. Sensitive rare cell analysis of CD34+ cells was performed using the MACSQuant Analyzer. In order to visualize pre-enriched human CD34+ cells from a peripheral blood mononuclear cell (PBMC) preparation a suitable gating strategy was devised (Figure 2.6: A to E). Cells were autolabeled with PE-conjugated anti-human CD34 antibodies to detect for CD34+ cells. Propidium iodide solution was used to exclude dead cells from flow cytometric analysis. An explanation of the gating strategy is given in the accompanying figure legend.

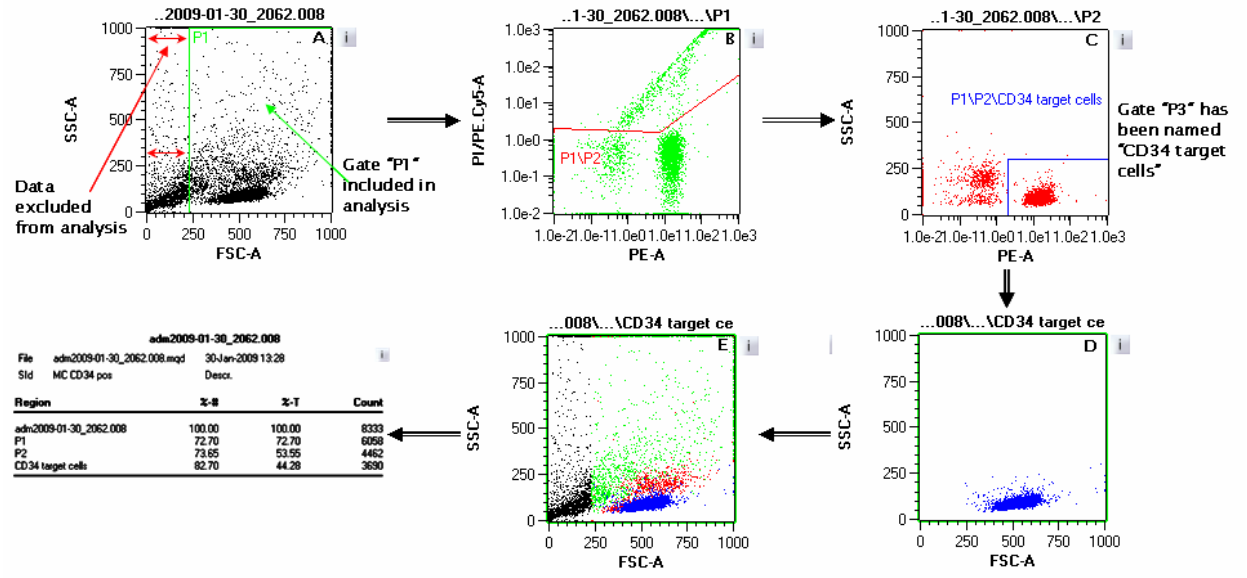


Figure 2.6 Gating strategy to profile CD34+ cells enriched from PBMC using the MACSQuant Analyzer.
A: P1 region was defined to remove dead cells and debris.
B: A region “P2” was defined within gate P1 to select for viable CD34+ cells. Any remaining dead cells are positive for PI and are excluded from the region P1\P2.
C: The region P3 was defined within gate P1\P2 to select for all viable CD34+ cells. Region P3 was renamed “CD34 target cells” for added clarity.
D: The final gate is displayed, namely: P1\P2\CD34 target cells. The corresponding statistics are shown by the adjacent table.
E: To demonstrate the gating strategy all defined regions were color-coded are display on a single two-parameter dot plot. P1 is green; P1\P2 is red; P1\P2\CD34 target cells is blue. The black dot plot events were excluded by region P1.

For a more detailed explanation on this gating strategy download the product data sheet “MACS Control: MC CD34 Stem Cell Cocktail, human” order number #130-093-427.

2.3 MACS® Cell Separation

MACS® Cell Separation, i.e. the magnetic separation of defined cell populations using MACS® Technology, is widely regarded as the gold standard in cell separation. MACS

Technology is based on the use of MACS MicroBeads, MACS Columns, and MACS Separators—strong permanent magnets. MACS Technology can be used for the targeted cell enrichment or depletion of cell types or populations through their expression of particular surface antigens. MACS Technology provides the means for the pre-enrichment of rare cells for subsequent flow cytometry analysis.

In a first step, surface antigens are magnetically labeled in a highly specific manner with monoclonal antibodies coupled to MACS MicroBeads. MACS® MicroBeads are superparamagnetic particles of approximately 50 nanometers in diameter, comparable to the size of a virus. MACS MicroBeads are not known to alter the scatter properties of cells in the flow cytometer or influence the light-microscopic appearance of the cell. They form a stable colloidal suspension and do not precipitate or aggregate in magnetic fields. MACS MicroBeads are composed of a biodegradable matrix made of iron oxide and polysaccharide; hence, it is not necessary to remove them after the separation process, saving hands-on time. MACS MicroBeads do not alter the structure, function, or activity status of labeled cells, and they are not known to interfere with subsequent experiments. Finally, MACS MicroBeads offer an extremely flexible tool for the pre-enrichment of many cell types from many species through the coupling of different antibodies. Several hundred reagents for the isolation of human, mouse, rat, and non-human primate cells, as well as reagents for indirect labeling of many other cell types, are available.

After magnetic labeling, the cells are passed through a MACS Column placed in the magnetic field of a MACS Separator. Non-labeled cells flow through and can be collected; labeled cells are retained in the column and can be released after removal of the column from the magnetic field. Thus, both labeled and non-labeled cell fractions can be efficiently isolated with MACS Technology. The entire procedure is fast, easy to handle, and gentle on cells, leading to the enrichment of cells that can be immediately analyzed.

The MACSQuant Analyzer is equipped with the MACS® Cell Enrichment Unit in order to provide a fast and easy way to analyze a rare cell population using MACS Technology. The MACSQuant™ Column, when properly seated in the MACS® Cell Enrichment Unit, can isolate up to 5×10^7 magnetically labeled cells. Upon separation of the magnetically labeled cells, the negative and positive fraction can automatically be analyzed. If pre-analysis enrichment is desired, cells can be labeled with MACS MicroBeads, separated using the MACSQuant Column, and then directly analyzed by flow cytometry in a fully automated fashion. Pre-enrichment of target cells by MACS Technology before fluorescence cell analysis is particularly valuable when target cells occur at extremely low numbers, such as stem cells or antigen-specific T cells.

2.4 Description of the MACSQuant® Analyzer

The MACSQuant Analyzer is a state-of-the-art benchtop cell analyzer for highly sensitive multicolor flow cytometry. Three lasers, combined with powerful MACSQuantify Software, make for a fast and simple analysis of cells. The instrument is equipped with two scatter (FSC, SSC) and seven fluorescence channels. Thus, the MACSQuant Analyzer is ideal for MACS Control applications (optimized evaluation of MACS Cell Separations) as well as standard immunofluorescence analyses. The system includes the MACS Cell Enrichment Unit that is crucial for the reliable detection and analysis of rare cells; it is directly controlled by the MACSQuant Analysis Software to enable the fully automated processing of samples and cell analysis. Automation can further be extended to multisample processing when combined with the MACS MiniSampler for convenient, hands-free operation.

Figure 2.7 Front image of the MACSQuant Analyzer – the access cover was made transparent for the purpose of illustration.

The MACSQuant Analyzer:

- Compact benchtop design
- Straightforward multiparameter cell analysis—from simple cell counting to sophisticated flow analysis
- Absolute cell counting (volumetric)
- Highly sensitive detection of rare cells
- Fully automated multisample labeling and analysis

3 Assembly and installation of hardware

The following section describes how the MACSQuant Analyzer is unpacked and installed for first use.

3.1 Unpacking the MACSQuant® Analyzer

Read through the following instructions carefully before commencing the installation procedure. Before opening the transportation box, check for any visible external damage to the box. Check also to see if the shock and position indicators (if present) suggest incorrect transportation of the instrument. If there is apparent damage please contact technical support for assistance (see section 13).

Name, description	Position in box
Fluid sensor cable module	Right at the bottom of the box
Power cable	Top storage compartment
Fluidics tubing (6×)	Top storage compartment
Bottle closures with sensors (4×)	Top storage compartment
MACS® MiniSampler with cover	Packaged in box located in upper storage compartment
Empty fluid containers with caps	In bottle holders
Single tube holder	Top storage compartment
Tube racks	Packaged in box located in upper storage compartment
MACSQuant® Analyzer	User manual, software DVD, keyboard and mouse

Table 3.1 Inventory and location of parts within transportation box.

1) Open the flight box or cardboard box (not shown) and remove the top layer of the packaging to reveal the instrument and associated packaging.

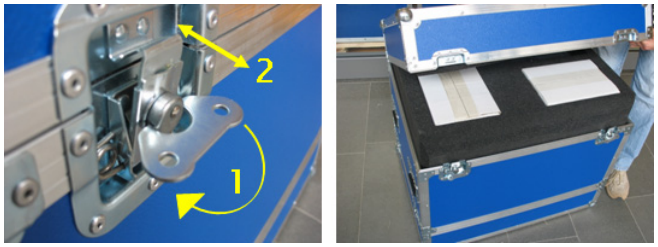


Figure 3.1 Opening the MACSQuant Analyzer flight-box. The lock handle must be turned (1) in order to release the lock-clip (2). The MACSQuant Analyzer may also be transported in a reinforced cardboard box.

Note: The top layer holds the MACSQuant Analyzer user manual, the MACS MiniSampler (when included) and various bags containing accessories. Carefully remove these parts.

2) Remove boxes containing the MACS MiniSampler and cover, and the accessories.

Figure 3.2 Packing format of the MACSQuant Analyzer and accessories.

3) Remove the foam packaging from both sides of the MACSQuant Analyzer.

Note: Two persons are required to lift the MACSQuant Analyzer. The instrument must be gripped at the base of the orange bottle baskets located at both sides of the device. Note that the instrument is heavier at the front. Ensure the front of the instrument is stabilized while lifting it.

Due care must be taken while lifting the MACSQuant Analyzer. Miltenyi Biotec accepts no liability for potential injuries sustained during lifting and/or movement of the device.

4) Place the instrument onto a stable worktop surface, e.g., laboratory bench.

Remove the plastic bag surrounding the device.

Note: Take into consideration that the instrument requires adequate air circulation for heat exchange and cooling. Refer to section 1.4.2 for elaboration.

5) Carefully remove the uptake port needle from the foam packaging.

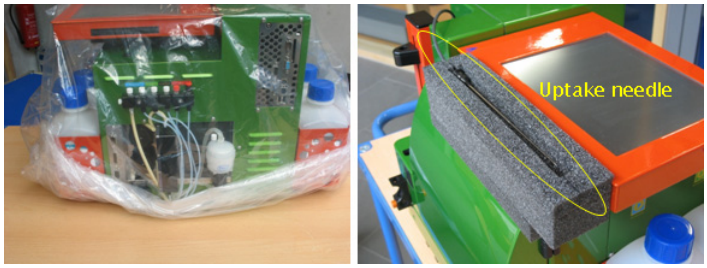


Figure 3.3 Left: The MACSQuant Analyzer was securely placed on stable worktop. Right: The plastic bag was removed. Note that the uptake needle is supported by foam.

6) Place the uptake port needle into its guiding at the needle arm.

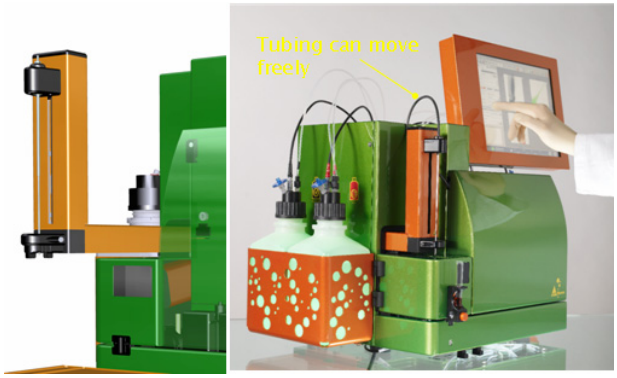


Figure 3.4 The needle port is positioned as illustrated above.

Note: Ensure that the tubing connected to the uptake port needle can move freely when the needle arm extends, or when the needle moves into the sample uptake position.

7) Adjust the angle of the touchscreen in an upright position in order to access the solution bottles and the fluidic ports on the back of the instrument.

3.2 Installation of the MACSQuant® Analyzer

Before installation, carefully read the chapter Important information (section 1).

The MACSQuant® Analyzer is a bench top instrument that fits neatly onto a benchtop and into standard sized laminar flow or safety cabinets. It should be installed on a stable, flat and vibration-free surface. The operating environment should be dust-free, sufficiently ventilated, and free from sources of electromagnetic radiation. In order to ensure a flat surface in laminar flow hoods, the MACSQuant® Analyzer can be placed on a MACS® Laminar Hood Plate (cat # 130-093-246).

Note: Before operating the MACSQuant® Analyzer for the first time, carefully read the user manual and contact your local Miltenyi Biotec representative for assistance.

Note: When delivered, the MACSQuant® Analyzer fluidics system is delivered dry i.e., without storage solution.

3.2.1 Connecting the fluid containers and fluid sensor cables

Operating the MACSQuant Analyzer requires running buffer, washing solution, and storage solution. Always operate the instrument with ready-to-use MACS Buffers and solutions. The MACSQuant Analyzer is delivered with four empty fluid containers (bottles) which can be found in the orange fluid container baskets connected to the instrument. The bottle closures consist of a fluid uptake port (for solutions: green, blue and black closures) or a fluid outlet port (waste container) as well as an electrolyte sensor for measuring liquid levels. The fluid containers, bottle closures, and fluid sensor cables are color coded for easier handling (see Table 3.2).

Container	Symbol	Container	Symbol
Running Buffer (blue)		Storage solution (black)	
Washing solution (green)		Waste (red)	

Table 3.2 Symbols and color coding of fluid containers.

1) Install one fluid at a time. Place a new bottle into the orange fluid container basket. Please note the corresponding color coding (see Table 3.2).

2) Unscrew the lid of the bottle and replace it with the appropriate bottle closures. Do not unscrew the fluid container lids until the bottle is placed in the basket.

3) Remove the fluid sensor cables and bottle closures from the packaging.

Blue-, green- and black-colored closures and accompanying hydrophobic filters in a single bag

Red-colored closure (waste) is packed in a separate bag

All bottle tubes packed in a single bag

Figure 3.5 Packaging format of the tubing, bottle closures (caps) and hydrophobic filters.

4) Remove the end-caps from the bottle distribution block.

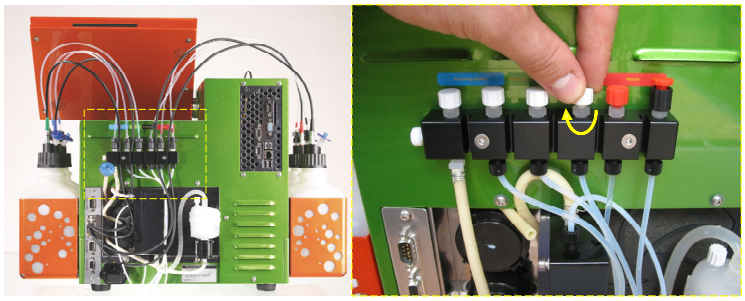


Figure 3.6 Before inserting the tubing into the fluid ports it is necessary to remove the end-caps from the bottle distribution block.

5) Connect the tubing to the appropriate color-coded fluid port on the back of the MACSQuant Analyzer.

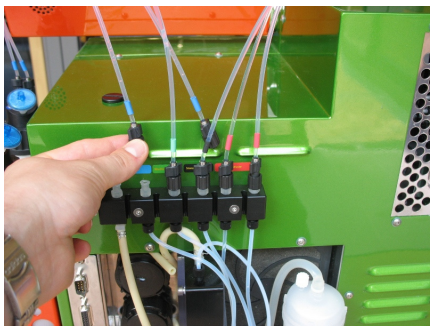


Figure 3.7 Connecting the tubing to the bottle distribution block.

6) Attach the sensor cable plug to the socket for sensor cables at the back of the MACSQuant Analyzer and fasten securely.

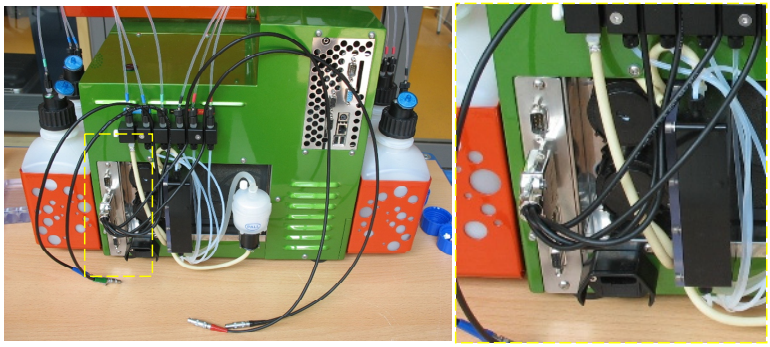
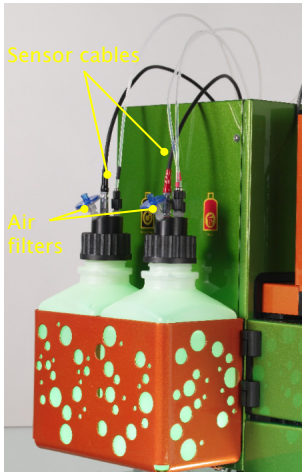


Figure 3.8 Connecting the sensor cables to the sensor cable port.

7) Note the color coding and connect each sensor cable to the respective bottle closure.



8) Connect the hydrophobic air filters (0.2 µm) to the appropriate connectors on the bottle closures.

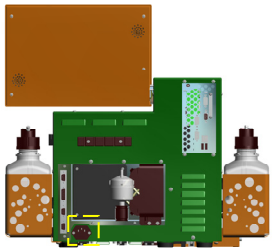
Note: The correct positioning of each solution container—recognizable by the color code and the symbols—is crucial for successful analyses using the MACSQuant® Analyzer.

To keep buffer sterile, each bottle closure should be equipped with a hydrophobic air filter. Avoid any contact of hydrophobic air filters with fluids as this may cause clogging of the filter.

When working with biohazardous samples, it is recommended to fill the waste container with 100 mL of disinfectant (e.g. MACS Bleach solution; order number #130-093-663) before use. For proper disposal, please follow local regulations and carefully read the chapter Important information.

3.2.2 Connecting the power cord

1) Note the position of the power socket on the rear panel of the MACSQuant Analyzer.

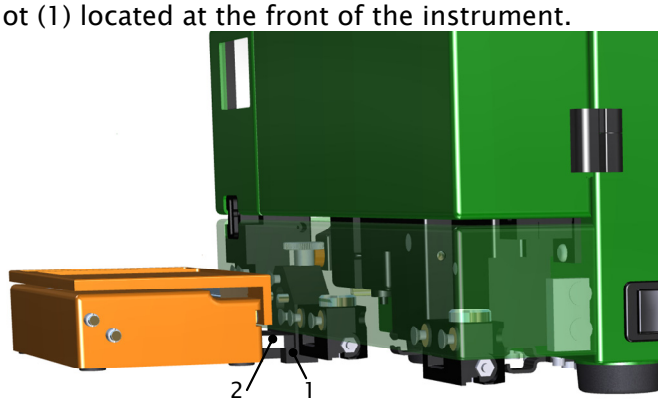


Ensure that the main power switch is in position “0” before connecting the power cord.

3.2.3 Installation of the MACS® MiniSampler and tube racks and reagent rack

The MACSQuant Analyzer is optionally delivered with the MACS MiniSampler, three different tube racks and a reagent rack (MACS Reagent Rack 4). Once installed, the MiniSampler is automatically recognized by the MACSQuant Analyzer. Each tube rack has a barcode on the rear side that is detected upon starting the separation process.

1) Remove the transparent protection foil from the lens of the rack detection. Note the positions of the MACS MiniSampler guiding (2) and its corresponding slot (1) located at the front of the instrument.



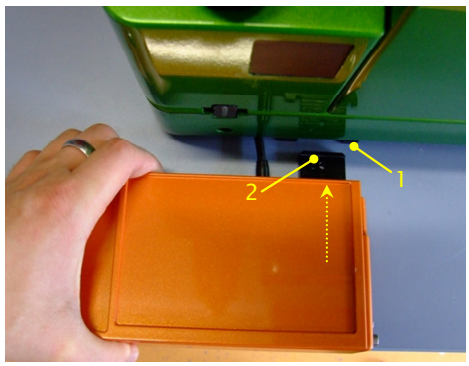


Figure 3.9 Location of the MiniSampler guide (2) and receiving slot (1) for the MACS MiniSampler.

2) Tilt the MiniSampler and slide the guiding into the receiving slot until resistance is met; lower the rack to a horizontal position i.e., the rack is locked in the position illustrated by the above figure.

3) Ensure that the MiniSampler is completely inserted and secured.

4) Note the position of the lid guiding at both sides of the MiniSampler and attach the lid.

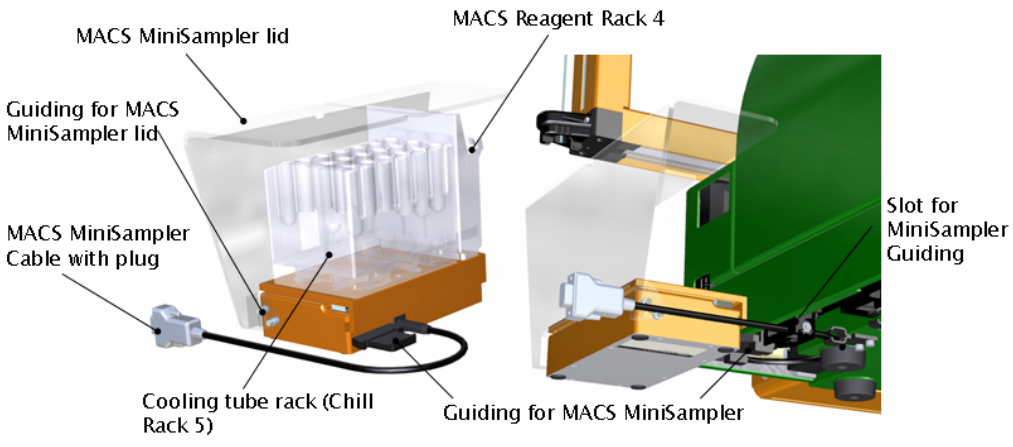


Figure 3.10 Rear view of MACS MiniSampler with MACS Reagent Rack and Chill Rack 5

5) Place the MiniSampler cable underneath the MACSQuant Analyzer and connect it to the socket (4) labeled "External CAN " at the rear panel of the instrument.

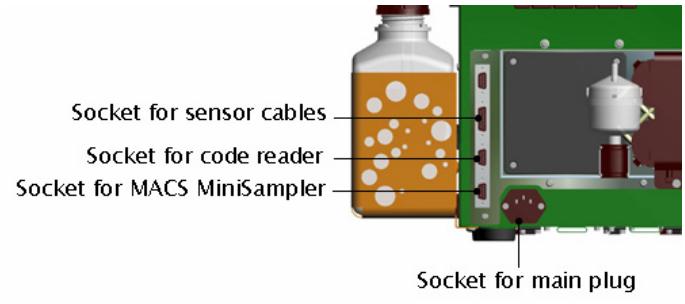


Figure 3.11 The MACS MiniSampler cable is attached to socket 4 at the back of the instrument.

3.2.4 Positioning of cooling tube racks and the MACS Reagent Rack 4

1) Open the lid of the MACS MiniSampler.

2) Secure the MACS Reagent Rack 4 onto the MiniSampler into the left recess. The engagement hook has to snap into the undercut.

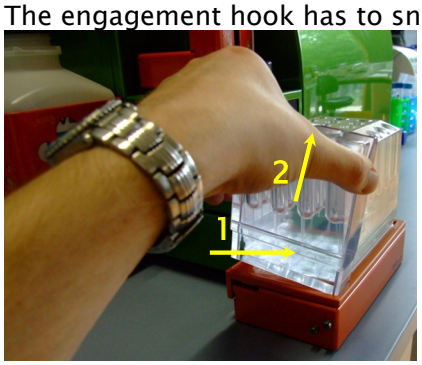


Figure 3.12 To remove the MACS Reagent Rack, gently press the rack in the planar direction "1" followed by lifting the rack in an upwards direction ("2").

3) Set a cool tube rack (e.g. Chill Rack 5) onto the MiniSampler into the right recess ensuring that the rack barcode is facing the MACSQuant Analyzer.

Figure 3.13 Positioning the Chill Rack 5 adjacent to a MACS Reagent Rack 4 on the MACS MiniSampler.

> **Note:** Racks can be pre-cooled for 3-4 hours at 2-8 °C. Do not cool below 0 °C since samples may freeze. If recognition of the tube rack fails, the instrument will display a screen for manual selection of the tube rack. Before confirming the choice, ensure that the rack is placed correctly into the recess.

3.2.5 Switching ON/OFF the MACSQuant® Analyzer

The main power switch is located on the right side of the instrument in front of the container baskets ("I" indicates "On", "O" indicates "Off"). Switch on the MACSQuant Analyzer.

Figure 3.14 Location of the on/off switch

3.2.6 Installation of the MACSQuant® Column (optional)

The MACSQuant Column can be ordered separately and can provide the flow user a fast and reproducible way to analyze rare cells without the necessity of long flow analysis times. The rare cell must be labeled with one of the MACS Microbead reagents as well as the fluorochromes of interest. This process in the presence of the MACS Enrichment Unit provide a flexible tool to pre-enrich a particular cell type prior to analysis.

> **Note:** These cells cannot be retrieved. Please refer to section 9.1.3 for more illustrated instructions on exchanging the MACSQuant Column.

Remove the column substitute and install the MACSQuant™ Column according to the following instructions.

1) Open the front door and note the position of the tubing and the pre-installed substitute (dummy) of the MACSQuant Column.

2) Using both hands, hold the top and bottom of the column substitute and pull gently but firmly to remove it from its slot in the MACS Enrichment Unit.

3) Place a paper towel under the column substitute. Hold the column substitute in one hand and gently unscrew the upper column connector anti-clockwise. Tilt the column substitute downwards to empty any fluid. Then unscrew the bottom column connector. Store the column substitute for later use.

4) Insert one end of the new MACSQuant Column into the bottom column connector and gently screw in the column by turning it clockwise until you feel resistance. Point the column towards the top of the device and screw in the top column connector.

> **Note:** The column has an appropriate orientation. The top portion of the column has a 3 mm filter in the end. This end must be in the upward position in order to achieve the best enrichment. See section 9.1.3 for more details.

5) Align the column so that the top column connector sits on the guide of the magnet cover. Press the column into the slot until you feel the guides click. Verify that the column is placed in the center of the magnet cover.

6) Close the front door.

3.2.7 Installation of the webcam

A webcam is provided for use with MACSQuant Live Support. See section 8 for details of how to contact Miltenyi Biotec technical support via MACSQuant Live Support.

> **Note:** A web-cam should be supplied with the MACSQuant Analyzer. If this is NOT the case, please contact your nearest MACSQuant Specialist.

To install the webcam:

1) Place the webcam in the holder assembly at the side of the instrument.

Figure 3.15 Location of the web-cam holder assembly.

2) Attach the camera USB cable to a free USB port at the back of the MACSQuant Analyzer

> **Note:** The web cam will automatically install. If this is not the case, please contact technical support or your local MACSQuant Specialist.

3.2.8 Installation checklist

The following checklist can be used to ensure that the MACSQuant Analyzer is correctly installed:

1) Ensure that the power cord is securely plugged into the MACSQuant® Analyzer and to a functional main power supply.

2) Ensure that all other tubing connections are fastened. If necessary, tighten loose connections using a wrench.

3) Ensure that the fluid containers are filled and installed correctly, that the correct tubing and fluid sensor cable is attached to the corresponding container, and that the bottle closures are fastened. Make sure that the waste bottle is empty.

> **Note:** When working with biohazardous samples, it is recommended to fill the container with 100 mL of disinfectant before use (e.g. MACS Bleach). For proper disposal, follow local regulations.

4) Optionally: Open the front door and check that the MACSQuant™ Column is installed correctly. Ensure that the tubes are securely fastened to the column and that no part of the visible tubing is pinched or obstructed.

5) When all points of this installation checklist have been fulfilled, close the front door.

6) Switch on the instrument using the power switch and pressing the touchscreen by hand.

3.3 Materials required for operation of the MACSQuant® Analyzer

The following section outlines the materials and consumables required for operation of the MACSQuant Analyzer.

3.3.1 Buffers and solutions

Running buffer, washing solution, and storage solution are required for daily operation of the instrument. Only use buffers and solutions supplied by Miltenyi Biotec for operation of the MACSQuant® Analyzer. A reproducible and optimal performance of the MACSQuant Analyzer cannot be guaranteed when the instrument is operated using self-made buffers and/or solutions procured from another manufacturer. Information for ordering MACSQuant™ Buffers can be found in Table 3.3.

Description	Color code	Capacity	Order no.
MACSQuant® Analyzer Running Buffer	Blue	6 × 1.5 L	130-092-747
MACSQuant® Analyzer Washing Solution	Green	6 × 1.5 L	130-092-749
MACSQuant® Analyzer Storage Solution	Black	6 × 1.5 L	130-092-748
MACSQuant® Starting Buffer pack		4x 1.5 L 1x1.5 L 1x 1.5 L	130-094-190
MACSQuant® Washing & Storage Solution Kit	n/a	3 × 1.5 L 3 x 1.5 L	130-092-801
MACS Bleach Solution	Black	6 × 1.0 L	130-093-663

Table 3.3 Running buffer and solutions for use with the MACSQuant® Analyzer

For safe operation of the MACSQuant® Analyzer, all fluid containers must contain at least 150 mL of the respective solutions and running buffer (except for the waste container). In order to prevent contamination of the fluidics system and the MACSQuant Column, the use of non-sterile buffers and solutions is not recommended.

MACSQuant Analyzer Running Buffer

The MACSQuant® Analyzer Running Buffer is a sterile filtered, ready-to-use buffer containing 0.09% azide as a preservative. The buffer is supplied in a 1.5 L container that can be connected directly to the MACSQuant® Analyzer.

MACSQuant Analyzer Washing Solution

The MACSQuant Analyzer Washing Solution is a sterile filtered, ready-to-use solution to rinse the fluidics system before the MACSQuant® Analyzer shutdown. It contains a detergent that dissociates cell aggregates and prevents the formation of plaques in the fluidics. The MACSQuant Analyzer Washing Solution was developed for an optimal cleaning of the MACSQuant Analyzer tubing system and is provided in 1.5 L container.

MACSQuant Analyzer Storage Solution

The MACSQuant Analyzer Storage Solution is a sterile filtered, ready-to-use solution used for the overnight or long-term storage of the MACSQuant Analyzer. The MACSQuant Analyzer Storage Solution is supplied in a 1.5 L container and prevents the corrosion or contamination of the fluidics system during long- or short-term storage of the instrument. Exchange of solutions within the fluidics system to the storage solution is performed automatically when the system shutdown protocol is activated in the software.

3.3.2 Hardware and disposables

The single-tube holder has been designed for compatibility with all standard flow cytometry tubes, 1.5 mL , 2 mL, and 5 mL tubes.

> **Note**: Should the MACS MiniSampler be used, the three tube racks available (Chill 5, Chill 15, and Chill 50) are designed to hold standard 5 mL, 15 mL, and 50 mL tubes, respectively (see Table 3.4). Additionally, the Chill 96 rack and the 96 rack can be used for the use of 96-well microtiter plates.

Rack type	Tubes	Maximum number of samples	Order numbers
Chill 5	5 mL	24	130-092-951
Chill 15	15 mL	15	130-092-952
Chill 50	50 mL	6	130-092-953
Chill 96/ 96 rack	96-well microtiter plate	96	130-094-459
Reagent rack	Reagent vials	4	130-094-574

Table 3.4 Rack types for the MACS® MiniSampler and compatible sample tubes

3.4 Materials required for maintenance of the MACSQuant® Analyzer

Solutions and hardware required for maintenance of the MACSQuant Analyzer are discussed below.

3.4.1 Solutions

Disinfectant solution
On spillage or splashing of sample, it is recommended to clean the port of the automated arm and the surface of the instrument with 70% ethanol or isopropyl alcohol and a dampened tissue. Alternatively, use alcohol swabs.

Distilled water
It is recommended to remove build-up of salt crusts with the use of a tissue dampened with distilled water.

3.4.2 Hardware

MACSQuant Columns
For pre-enrichment of rare cells prior to analysis. The MACSQuant™ Column has a capacity of 5×10^7 magnetically labeled cells. The column should be replaced every three months.

Column substitute
For installation prior to storage of the MACSQuant® Analyzer when storing for longer than three months. The instrument is delivered with column substitute installed.

Hydrophobic 0.2 µm air filters
Hydrophobic air filters are used to vent fluid bottles and maintain sterility. Do not use hydrophilic filters, since they are easily blocked upon contact with liquid.

Pre-filter
The pre-filter is designed to prevent particles (salt crystals etc.) from entering the fluidics system. When installing the instrument, the white lid on the top of the filter may need to be unscrewed to bleed the system.

3.5 Calibration of the MACSQuant® Analyzer hardware

When using the MACSQuant Analyzer for the first time it is necessary to calibrate the hardware before calibration of the instrument settings. This section discusses hardware calibration.

Note: It is highly recommended that hardware calibration is only performed by an administrator who has been trained by Miltenyi Biotec.

Note: In order to calibrate of the MACSQuant Analyzer the user must be familiar with the MACSQuantify Software. Refer to section for an introduction to the MACSQuant Analyzer user interface.

3.5.1 Calibration of the uptake unit

1) Click on the **Tools** tab and **Calibrate uptake unit** box.

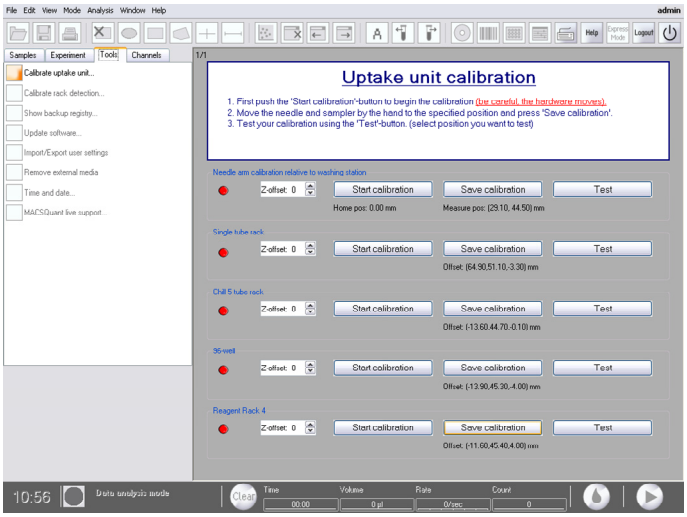


Figure 3.16 Selection of Tools tab for calibration of uptake unit

Note: The red closed circles shown on Figure 3.16 indicate that all four uptake components (Needle arm, Single tube rack, Chill 5 tube rack, 96-well plate, Reagent Rack 4) are not calibrated.

Note: In order to meet the minimal hardware calibration requirements of the MACSQuant Analyzer, the position of the needle arm in relation to the washing station and single tube rack must be calibrated. THIS IS A MINIMUM REQUIREMENT.

Calibration of needle arm

The needle arm moves between the samples and sample injection port/needle wash station along the y and z axis. It is imperative that the arm is correctly calibrated.

1) Ensure that the needle arm can freely move and that no object is obstructing it.

2) Click **Start calibration** under the heading **Needle arm calibration relative to washing station**. The needle arm will move toward the wash station (y-axis) before being inserted into the sheath flow port (z-axis).

Note: If the calibration unit is grossly misaligned, the needle may pop-out of the needle-arm holder. This will cause a system failure, but will not damage the resulting components. Simply reinsert the needle into the holder and adjust the needle arm appropriately. A dialog box will appear to reinitialize, press ok.

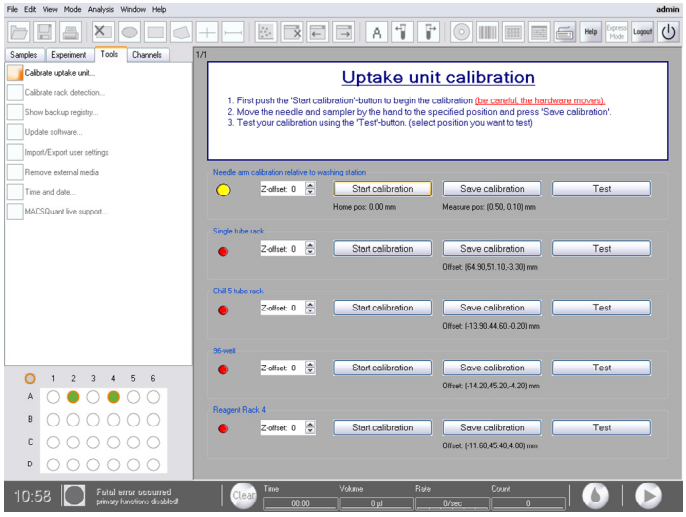


Figure 3.17 Calibration of the uptake unit is underway. The yellow closed circle indicates that the needle arm may be freely moved for manual calibration.

3) Manually adjust the y-axis of the needle arm by moving it towards you, taking care to ensure that the needle is correctly positioned over the center of the sheath flow port.

4) Gently lower the needle arm into the sheath flow port until the needle makes first contact with the bottom of the orifice. The needle should always remain perpendicular to the horizontal plane, i.e., should not be arched or inserted diagonally.

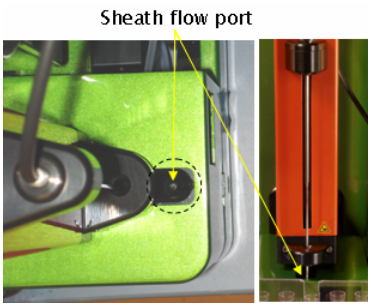


Figure 3.18 Inserting the needle arm into the sheath flow port.

5) Click **Save calibration**.

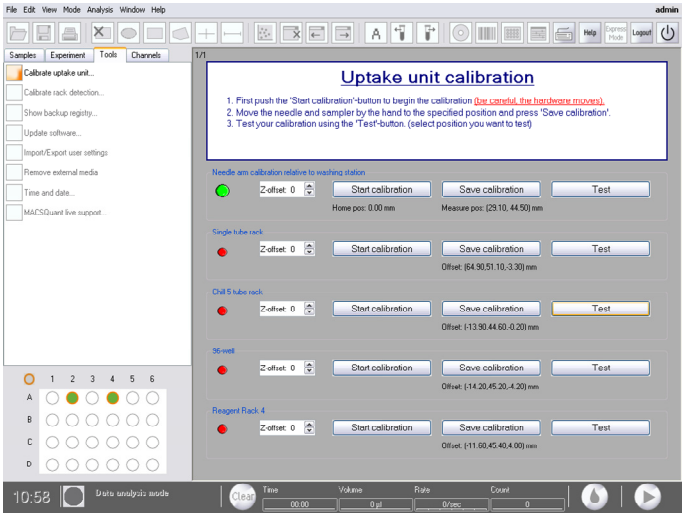


Figure 3.19 Coordinates of the needle arm are saved.

6) The closed green circle indicates that calibration of the needle arm position is completed.

7) Click **Test** to confirm that the correct coordinates are saved.

Calibration of the single tube rack

1) Gently insert the single tube rack it into the corresponding slots located at the front of the instrument (position 5, Figure 3.20). The rack should 'click' into place.

Figure 3.20 The single tube rack is attached to the instrument as illustrated above.

2) Click the **Experiment** tab and select the **Single tube rack** format from the **Rack** pull-down menu. Note: If the adjacent checkbox is activated (☑) the rack will be detected automatically.

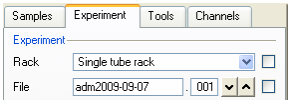


Figure 3.21 Selecting a "single tube rack" for rack calibration

3) Click **Start calibration**. The needle arm will automatically move forward and insert the needle into the single tube.

   The needle should be positioned as follows:

   i) At the center of tube on the y-axis (i.e. equidistant from the tube edges).

   ii) Only a fraction of a millimeter from the bottom of the tube (z-axis) i.e. almost touches the tube bottom. To check the needle position, gently wiggle the tube to ensure that there is a small amount of movement.

   If this is not the case, carefully adjust the needle arm accordingly.

   When satisfied with the needle position, click **Save calibration**.

4) A successfully completed procedure is indicated by a green closed circle.

5) Click **Test** to confirm that the correct coordinates are saved.

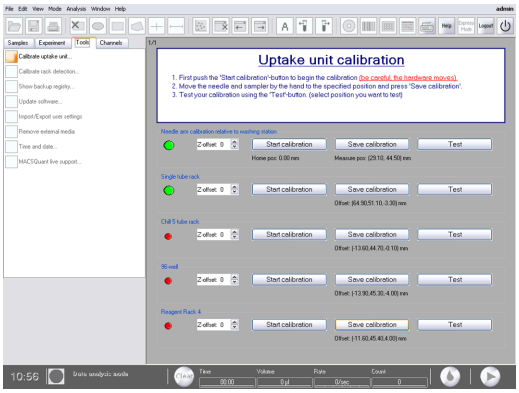


Figure 3.22 Green closed circles indicate successful calibration of the needle arm and single tube rack

Calibration of chill racks (recommended if a MACS MiniSampler is attached)

1) Ensure that the MACSQuant MiniSampler is correctly attached to the instrument. This includes fastening of the corresponding cable to the **External** CAN port located at the back of the instrument (see section 3.2.3 for information about correct connection of the MACS MiniSampler).

2) Click the **Experiment tab** and select the **Chill 5 tube rack** format from the Rack pull-down menu.

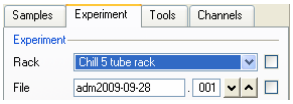


Figure 3.23 Selecting Chill 5 tube rack using the Experiment tab

Note: The Chill 15 and Chill 50 rack do not need to be calibrated separately. The calibration of the Chill 5 will also ensure for the appropriate calibration of the Chill 15 and Chill 50 rack.

Note: If the Chill rack 5 is not selected the following error will be reported: **"Specified rack must be specified in the experiment settings".**

3) Load a Chill 5 rack with empty 5 mL tubes in the position D6 (see Figure 3.24).

Figure 3.24 Position D6 (marked 'X') is used for calibration of Chill Rack 5.

4) Place the loaded rack onto the MACS® MiniSampler.

5) Click **Start calibration**.

The needle arm will automatically insert the needle into a tube located at rack position D6.

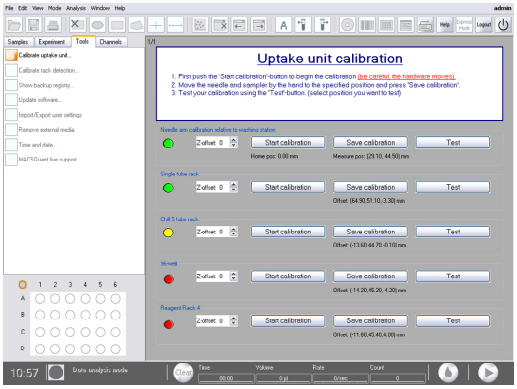


Figure 3.25 Calibration of the uptake unit is underway. The yellow closed circle indicates that the needle arm may be freely moved for manual calibration.

6) The needle should be positioned as follows:

i) At the center of tube on the y-axis (i.e. equidistant from the tube edges).

ii) Only a fraction of a millimeter from the bottom of the tube (z-axis) i.e. almost touches the tube bottom. To check the needle position relative to the bottom of the tube, gently wiggle the tube to ensure that there is a small amount of movement.

If this is not the case, carefully adjust the needle arm accordingly.

7) When satisfied with the needle position, click **Save calibration**.

8) A successfully completed procedure is indicated by a green closed circle.

9) Click **Test** to confirm that the correct coordinates are saved. The MACSQuant Analyzer will automatically test the remaining sample positions.

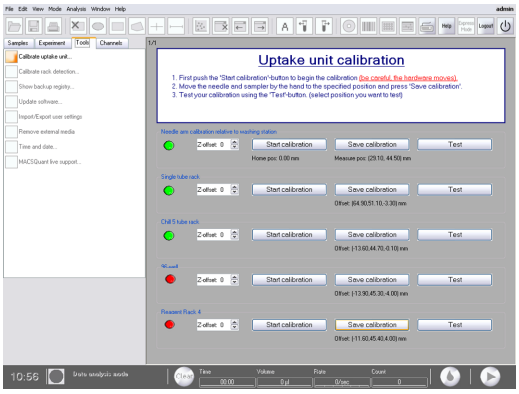


Figure 3.26 Chill 5 tube rack is successfully calibrated

Calibration of MACS® Chill 96-well plate (optional step)

1) Ensure that the MACS® MiniSampler is correctly attached to the instrument. This includes fastening of the corresponding cable to the External CAN port located at the back of the instrument (see section 3.2.3 for information about correct connection of the MACS MiniSampler).

2) Click the **Experiment** tab and select the **96-well** format from the **Rack** pull-down menu.

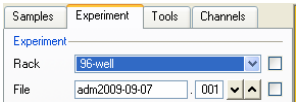


Figure 3.27 Selecting a 96-well rack for rack calibration: ensure the checkbox is activated

3) Place an empty 96-well plate on one of the 96 racks and place both onto the MACS MiniSampler.

4) Click **Start calibration**.

The needle arm will automatically insert the needle into a rack position, H12, specified by the MACSQuantify Software.

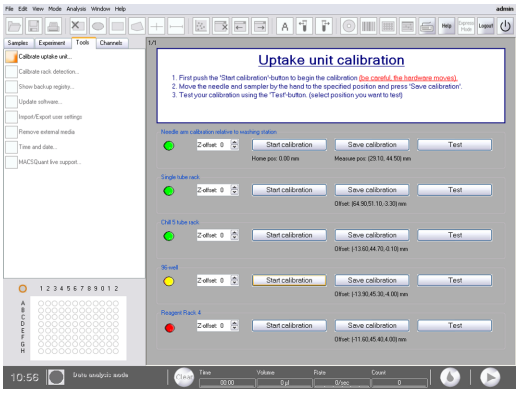


Figure 3.28 Calibration of the 96-well rack is underway. The yellow closed circle indicates that the needle arm may be freely moved for manual calibration.

5) The needle should be positioned as follows:

i) At the center of well on the y-axis (i.e. equidistant from the plate well edges).

ii) Only a fraction of a millimeter from the bottom of the plate well (z-axis) i.e. almost touches the bottom of the plate. To check the needle position relative to the bottom of the plate well, gently wiggle the plate to ensure that there is a small amount of movement.

If this is not the case, carefully adjust the needle arm accordingly.

6) When satisfied with the needle position, click **Save calibration**.

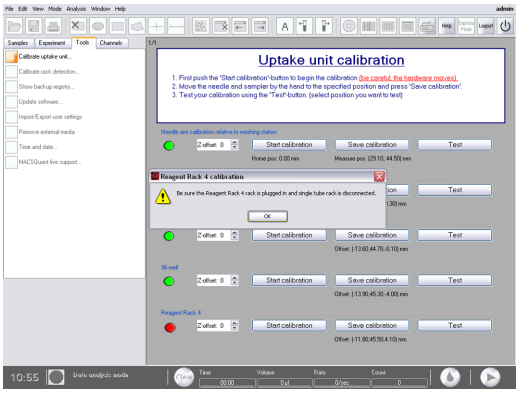
7) A successfully completed procedure is indicated by a green closed circle.

Calibration of MACS® Reagent Rack 4

1) Ensure that the MACS® MiniSampler is correctly attached to the instrument. This includes fastening of the corresponding cable to the External CAN port located at the back of the instrument (see section 3.2.3 for information about correct connection of the MACS MiniSampler).

2) Ensure that the MACS Reagent Rack 4 is correctly placed on the MiniSampler.

3) Click **Start calibration**. A dialog box will prompt you to ensure that the Reagent Rack is indeed correctly placed and that the single tube rack is removed. If this is the case, click **OK**.



4) The needle arm will automatically be positioned in reagent vial position A1. If the position is correct, click **Save calibration**.

5) Click **Test** to test the calibration. The needle arm should be correctly positioned in all four vials.

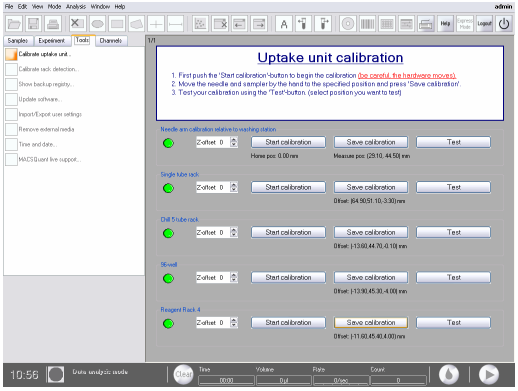


Figure 3.29 All uptake units have been correctly calibrated.

3.5.2 Calibration: rack detection

Single tube rack and acquisition button detection

1) Click on the **Tools** tab and **Calibrate rack detection**.

2) Click **Start calibration.**

   The dialog box will prompt you to remove the single tube holder from the front of the instrument.

3) Remove the single tube holder by pinching the single tube rack using both index fingers (the orange button is used only for acquisition; it does not release the single tube rack from the instrument). The single tube rack can be easily removed by gently pulling the component away from its docking-adaptor.

Figure 3.30 Removing he single tube holder. The orange button is for acquisition only.

4) The software will then direct you to re-connect the single tube rack.

   This is performed by inserting the two male-pins of the single sample rack into the female docking adaptor until its 'clicks' into position.

5) Lastly, the software will prompt you to press and hold the orange sample acquisition button until the calibration is completed. A successful calibration is

indicated by a green closed circle.

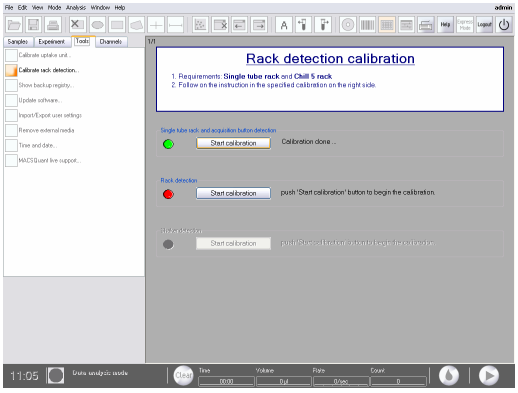


Figure 3.31 Successful completion of single rack detection

> **Note:** For the rack calibrations, the user must perform the prompted tasks promptly in order to calibrate the racks properly. Otherwise, the calibration will fail.

Rack detection

This step is required for the automatic detection of Chill 5, 15, and 50 mL tube racks by the MACS® MiniSampler.

1) Ensure that the MACS® MiniSampler is correctly attached to the instrument.

   This includes fastening of the corresponding cable to the **External CAN** port located at the back of the instrument.

> **Note:** The single tube rack must be disconnected before performing this procedure. Ensure that no objects are obstructing the MACS MiniSampler as this component will move during calibration.

2) Select the Chill 5 rack from the **Experiment** tab and **Rack** dropdown menu.

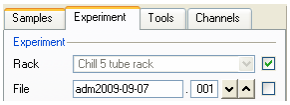


Figure 3.32 The rack checkbox, ☑, must be activated in order to perform rack detection

3) Click **Start calibration** of the Rack detection.

4) Place one Chill 5 tube rack onto the MACS MiniSampler.

   The MACSQuant Analyzer will immediately move the Chill rack to and fro while checking the barcode reader for operability.

5) A successful calibration is indicated by a green closed circle and the text report **Calibration done**.

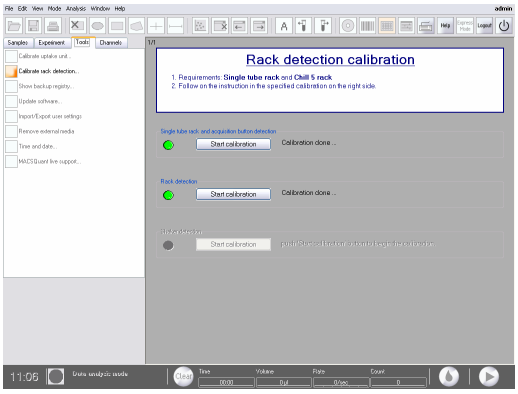


Figure 3.33 Rack detection is successfully completed.

3.6 Calibration of the instrument settings

In flow cytometry, fluorescence intensity is used to distinguish between 'positive' and 'negative' populations of particles. The reproducibility and stability of the fluorescence signal over time is of vital importance. In order to ensure a stable measurement that is independent of time and the specific analyzer, instrument calibration is performed. Fluorescence calibration curves are calculated by using standardized fluorescence microbeads that have predefined sizes and fluorescence intensities. A linear regression equation is calculated from the instruments response in mean or modal histogram channel values to these predefined values.

3.6.1 Performing a fully automated calibration

1) Prior to beginning calibration, ensure that the single tube holder is correctly attached.

Figure 3.34 The single tube holder and orange acquisition button.

2) Activate the reader by clicking on the **Barcode** icon ( ) and present a vial of MACSQuant™ Calibration Beads in front of the barcode reader. To proceed with the calibration process, select **Yes.**

3) Follow the dialog box instructions i.e., place an empty tube into the **single tube holder** and dispense one drop of MACSQuant Calibration Beads into it. Note: Ensure that you mix the calibration beads prior to dispensing.

4) Click **OK** to commence the calibration process. The uptake needle will dilute the calibration beads to a total volume of 0.5 mL, following which 100 µL will be taken up and injected into the flow sheath port for the calibration procedure. During calibration the gain and trigger for each respective channel will be automatically adjusted. The MACSQuant Calibration Beads consist of two sizes of beads (2 µm unstained beads and 3 µm beads stained with fluorochromes to emit fluorescence in all 7 channels). For more information about the MACSQuant Calibration Beads, please review the product data sheet available at www.miltenyibiotec.com.

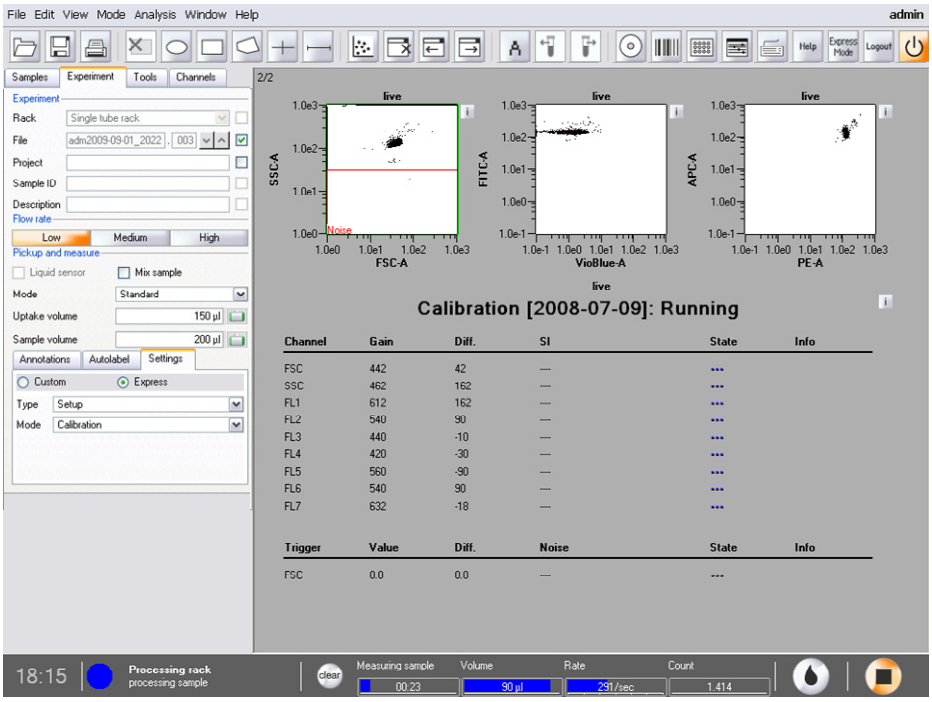


Figure 3.35 Calibration is underway.

When the process is successfully completed, the MACSQuant Analyzer **Status bar** should report *MACSQuant ready: Calibration OK*. These settings will be automatically saved as the default settings.

5) The calibration results for each channel are presented as dot plots, histograms and as a tabulated summary on a two-page (two-screen) report. Successful calibration for each channel is indicated by a green checkmark. To view all

calibration dot plots and histograms click **Next screen**,  or **Previous screen**  .

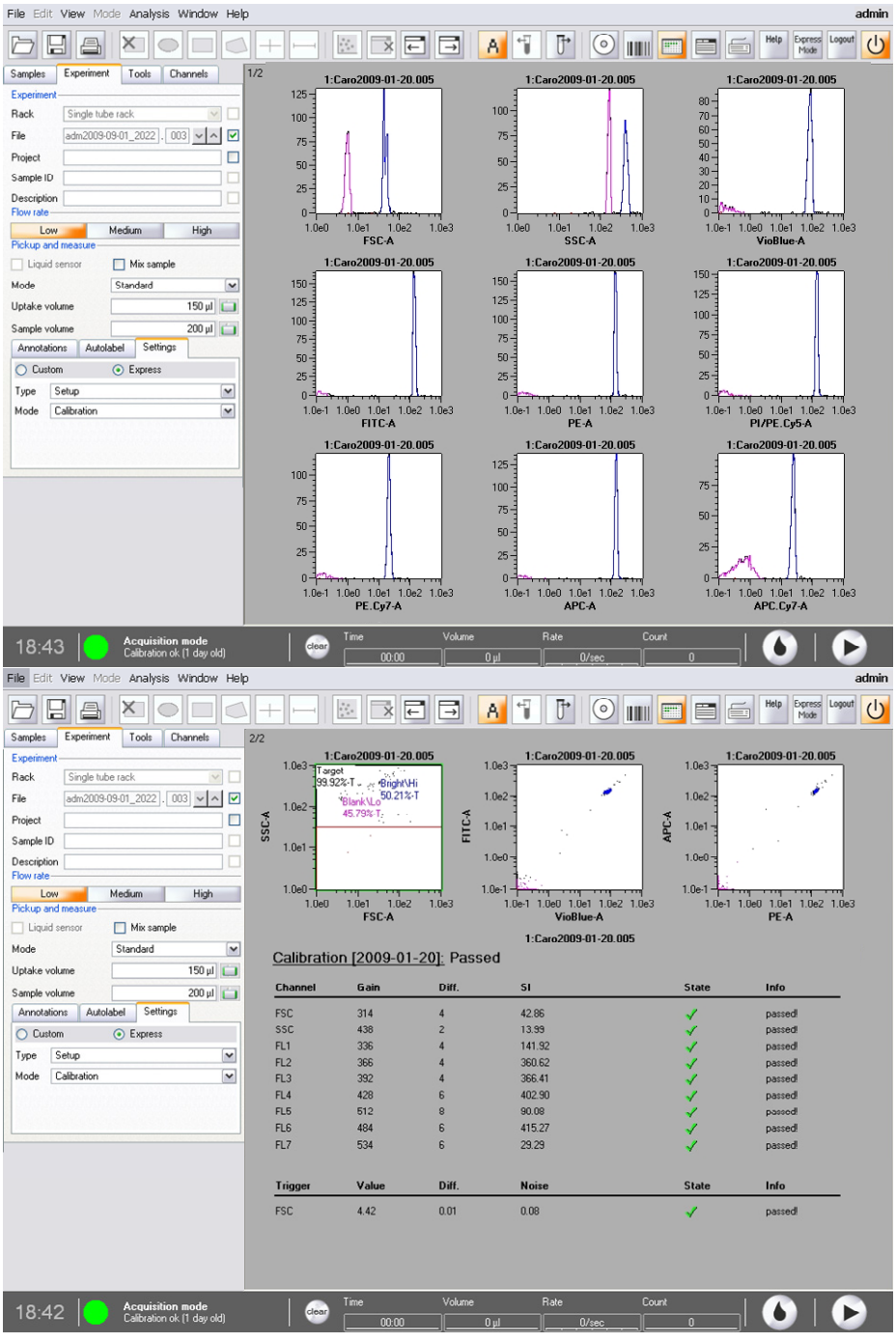


Figure 3.36 Successful calibration of the MACSQuant Analyzer as shown by an array of histograms (upper) and associated summary table (lower).

3.6.2 Performing manual calibration

Custom mode users and administrators can perform manual calibration as follows.

> **Note:** The calibration beads must be pre-diluted and mixed before performing this procedure. The MACSQuant Analyzer can perform pre-dilution and mixing of calibration beads; see section "Setting the dilution and mixing of the calibration beads prior to calibration" for more details.

1) In the **Custom** mode, select the **Experiment** tab on the left side of the screen.

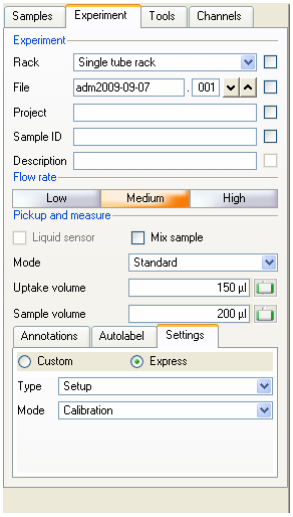


Figure 3.37 Setting-up calibration in Custom mode using the Express option (radio button).

2) Select the **Settings** tab in the lower section of the panel and click on the **Express** radio button.

3) Click the **Type** pull-down list and select **Setup.** Similarly, choose **Calibration** from the **Mode** pull-down list.

4) Click on the **Start Measurement** button,  .

This will start the calibration process.

Setting the dilution and mixing of the calibration beads prior to calibration

Dilution of the calibration beads can be performed by the MACSQuant Analyzer.

1) Select the Autolabel tab within the Experiment tab.
2) Click <**add**...>. This will introduce a Reagent dialog box.
3) Select S1 and prebuffer and adjust the dilution appropriately. The buffer should be set at 10:1 with no incubation time.

4) Follow the steps described for (manual calibration).

3.7 Compensation of the instrument settings

A proper compensation of the instrument is crucial for the optimal acquisition and display of data obtained by flow cytometry. Compensation essentially accounts for the inherent overlap in emission spectra observed between different fluorochromes. This fluorescence spectral overlap, or 'spillover', will result in the detection of individual fluorochromes in more than one fluorescence channel. This overlap should be determined and corrected. This is especially important when multicolor analyses are to be performed—without proper compensation, results may be misinterpreted.

For example, FITC fluorescence will be detected in both the FL-2 (FITC) and FL-3 (PE) channels, though FL-2 is the designated channel for FITC detection. Therefore, all FITC fluorescence signal detected in FL-3 is regarded as excess or 'spillover'; this excess is calculated as a percentile, the value of which is mathematically 'subtracted' (compensated) from the original signal. This is best displayed by Figure 3.38 where cells labeled with a CD8-FITC antibody are measured in an FL-2 vs. FL-3 dot plot. The left panel shows an uncompensated detection of the cells, where $CD8^+$ cells are detected in both the FITC channel (FL2) and the PE channel (FL3) and hence appear to be double-positive for FITC and PE fluorescence even though they have only been stained with a FITC-conjugated antibody. After compensation (right panel), the percentage overlap between the two channels has been removed, ensuring that FITC signal from $CD8^+$ labeled cells are restricted to the x-axis (FITC).

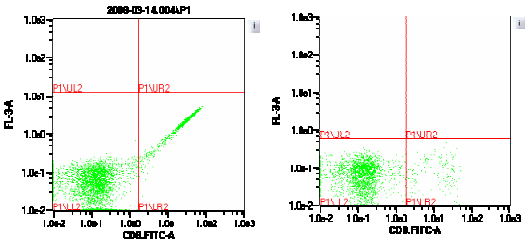


Figure 3.38 Cells stained with CD8-FITC were optimally compensated (right) for FITC detection in the FL3 channel

> **Note:** It is not possible to subjectively estimate what fraction of the signal needs to be subtracted; compensation settings must be individually optimized for all activated fluorescence channels as well as for each fluorochrome and fluorochrome combination used. An optimal compensation is of particular importance to distinguish dimly labeled cells from a negative population.

3.7.1 Performing auto-compensation

Compensation should only be performed if the instrument has been successfully calibrated and when the MACSQuant Analyzer reports a *MACSQuant ready: Calibration OK* status. Automated instrument compensation is based on a 7×7 matrix. It is

recommended that the MACSQuant Analyzer automatically calculates the appropriate compensation settings by using the automated compensation matrix and the compensation program (matrix method). However, more advanced flow users may wish to adjust these settings manually using sliders (classic method).

Using either single-stained cells or compensation beads, the MACSQuant Analyzer can accurately and reliably perform automated compensation based on a 7x7 matrix (each channel compensated against the other 6 channels). The utilized cell type and its respective surface marker should be brightly expressed on cells. For example, control sample aliquots of a peripheral blood mononuclear cell (PBMC) preparation are often singly-stained using CD8-FITC, CD8-PE or CD8-APC antibodies, in order to compensate fluorescence signals in FL2, FL3 and FL6.

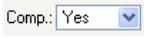
Using individually stained live cells for compensation

1) Label the cells individually using appropriate fluorochromes that correspond to the fluorescence channels of interest. Immediately prior to measurement transfer equal volumes of all single-stained cell aliquots, including a negative sample into one tube and place in front of the MACSQuant Analyzer.
   For further information concerning available reagents and corresponding staining protocols, please refer to the Miltenyi Biotec catalog and/or datasheets at www.miltenyibiotec.com and www.macsquant.com.

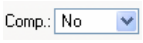
2) Select the fluorescence channels that require automatic compensation.
   From the menu bar select **Edit** and **Calibration** (or Ctrl+Alt+C) to open the **Calibration settings**... dialog box.
   Select which channels require compensation using the **Comp.** drop-down list (based on the following nomenclature):

   : fluorochrome particle (P) in this channel.

   : no fluorochrome particle in this channel, but the amount of fluorescence 'spillover' into this channel needs to be determined.

   : no fluorochrome particle in this channel, and the compensation value in this channel will not be determined.

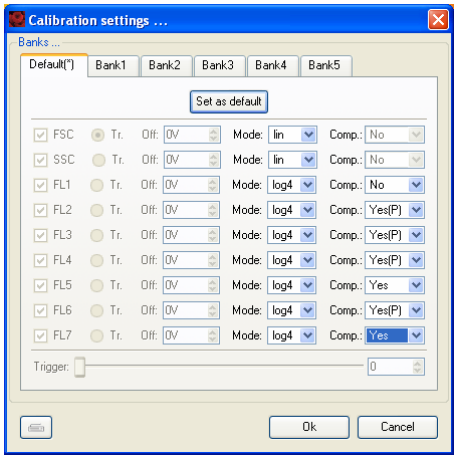


Figure 3.39 Selecting channels for compensation

In the above example a 4-color calibration was performed which comprised fluorochromes to test the following primary signals:

Fluorescence-2 (FL2): FITC (yellow-green)
Fluorescence-3 (FL3): PE (red-orange)
Fluorescence-4 (FL4): PE.Cy5.5 (red)
Fluorescence-6 (FL6): APC (blue-green)

No fluorochrome was included for evaluation of the FL5 channel (PE.Cy7). Compensation is nevertheless desired for this channel as indicated by Comp.: Yes.

3) Instruct the instrument to perform automatic compensation from the **Experiment** tab. Select the **Settings** tab in the lower section of the left panel and click on the **Express** radio button.

Click on the **Type** pull-down list and select **Setup.**

Similarly, choose **Compensation** from the **Mode** pull-down list.

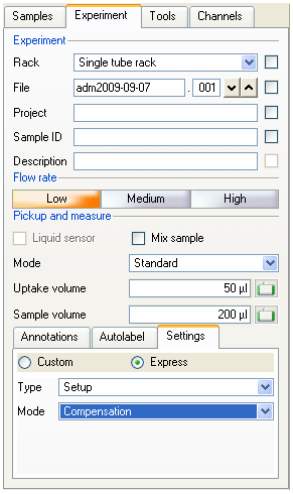


Figure 3.40 Setting up compensation from Custom mode using the Express radio button.

4) Click on the **Start Measurement** button ( ).

This will start the automated compensation process. A dialog box will appear to describe that compensation will only be performed on the default settings. An analysis template is automatically generated in **Live** mode for each of the previously selected channels. Initially, the MACSQuant® Analyzer acquires 2,500 events before determining the compensation values for the channels, the instrument will continue to acquire events until all of the channels have been compensated. All newly acquired settings for the compensation are automatically saved in the compensation matrix.

5) The newly saved instrument settings will automatically be saved into the current default settings. The calibrated and compensated settings are now saved as the default settings for the day's date.

6) Save the instrument settings either to a **Public** or **Private** folder.

Only save the instrument settings if the compensation was correctly performed. Select **File** and **Save** from the menu (Ctrl+S). Highlight **Instrument settings** on the left navigation bar; enter the filename in the **Setting** field and **Save** the settings. The file is automatically saved.

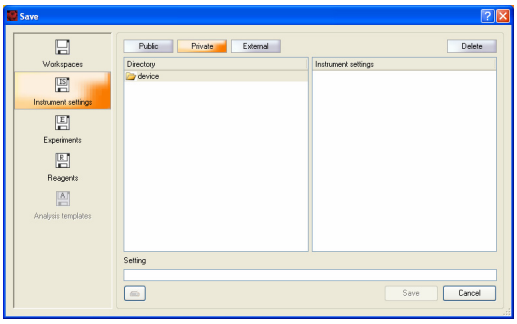


Figure 3.41 Saving instrument settings. Users with sufficient administration rights can save work to public or private folder.

Performing manual compensation

Many experienced flow users prefer to set the compensation settings manually. This can easily be accomplished by performing a standard measurement and adjusting the compensation values using the **Classic** or **Matrix** compensation menu.

1) First, perform automated compensation: see section 3.7.1for more details.

To access the compensation settings and change them manually:

2) Open the **Edit** menu, select **Instrument settings...**, and then click on the **Compensation** tab.

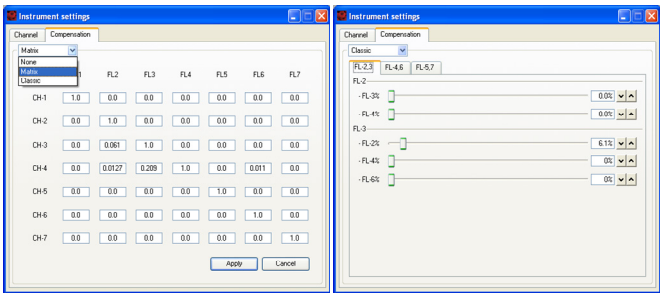
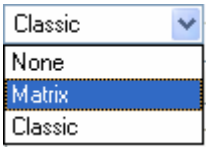


Figure 3.42 Two compensation modes can be selected from the Instrument Settings compensation dialog box: Matrix (left) and Classic (right).

3) Use the drop-down list to toggle between **Matrix** and **Classic** modes.



Note: Converting data from **Matrix** to **Classic** formats is not lossless, i.e., **all** changes made to the matrix may not be completely converted to the classic format. The user should take care to ensure that manual compensation settings are always verified using acquired data, i.e., labeled cells or compensation beads.

Note: It is recommended to use the matrix for compensation.

4) Reset the matrix by selecting **Classic** and then select **Matrix**.

5) Change the matrix values to adjust cell populations as required. See the following example for further clarification.

An example of single color compensation: PE fluorescence measured in the FITC channel

In the following example human peripheral blood cells were stained with R-Phycoerythrin (PE)-conjugated and Fluorescein-conjugated (FITC) monoclonal antibodies. PE and FITC and are both excited by the MACSQuant Analyzer blue laser operating at the wavelength 488 nm. The emission spectra from each fluorochrome are detected by two discrete photomultiplier tubes (PMT): FITC by CH2, and PE by CH-

3. Nevertheless, there is unavoidable cross-over of FITC fluorescence (FL2) in the PE recording channel (CH-3) and simultaneous cross-over of PE fluorescence (FL3) in the FITC recording channel (CH-2). To correct for this so called fluorescence 'bleed' instrument compensation is necessary, see Figure 3.43 for illustration.

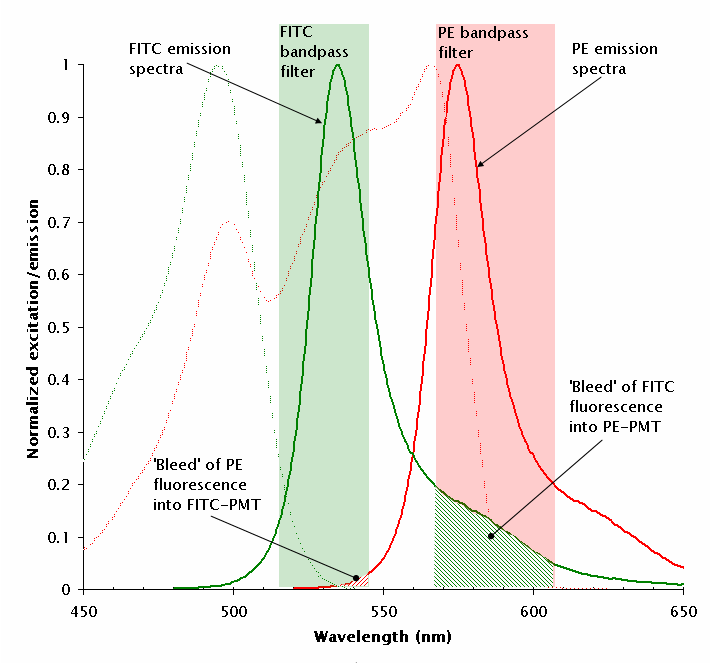


Figure 3.43 Excitation (dashed lines) and emission spectra (continuous lines) of Fluorescein (FITC) and R-Phycoerythrin (PE). The wavelengths of the corresponding band-pass filters used to restrict PE and FITC fluorescence to the respective FL2 and FL3 channels are also shown. In spite of the use of appropriate band-pass filters, 'contamination' of FITC fluorescence in the PE recording channel (CH-3) is apparent. The converse is also true: i.e. 'bleed' of PE into the FITC channel (CH-2). In flow cytometry the 'contaminating' signal or 'bleed' is electronically subtracted by performing instrument compensation.

The original uncompensated density blot and corresponding compensation matrix for the acquired data is shown in Figure 3.44. PE bright cells, denoted by population A, show a notable fluorescence signal in the FITC channel (CH-2). This should not be the case; population A must move in a downward direction so that the median FITC-A fluorescence intensities are identical to PE-dim and PE-bright cell populations.

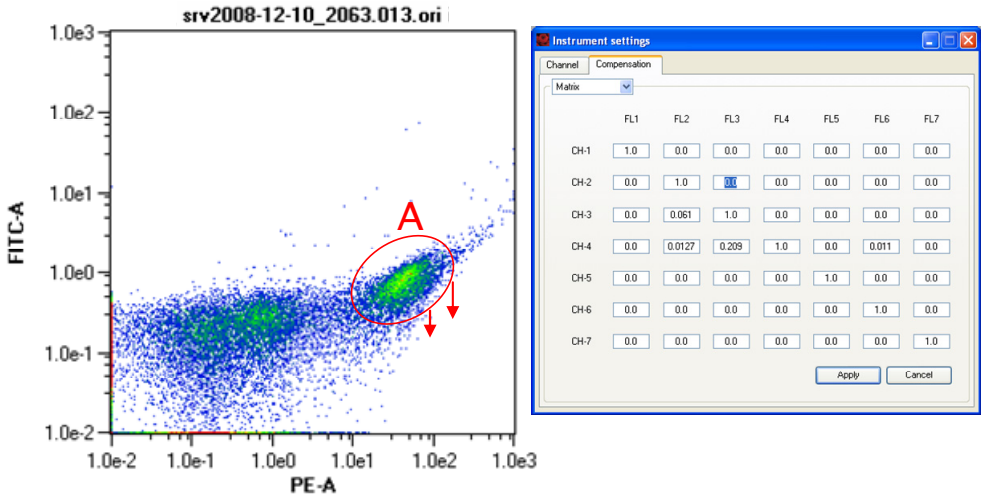


Figure 3.44 Uncompensated data density plot and corresponding compensation matrix. Population A is composed of bright PE-stained cells which show a significant fluorescence signal in the FITC channel (CH-2). The corresponding value in the compensation matrix is 0.0, i.e., no compensation was performed.

To compensate for 'bleed' of PE fluorescence (FL3) in the FITC channel (CH-2) the compensation value for the matrix coordinate "FL3/CH2" must be increased. In this case the value was increased from 0.0 to 0.012 (see Figure 3.45).

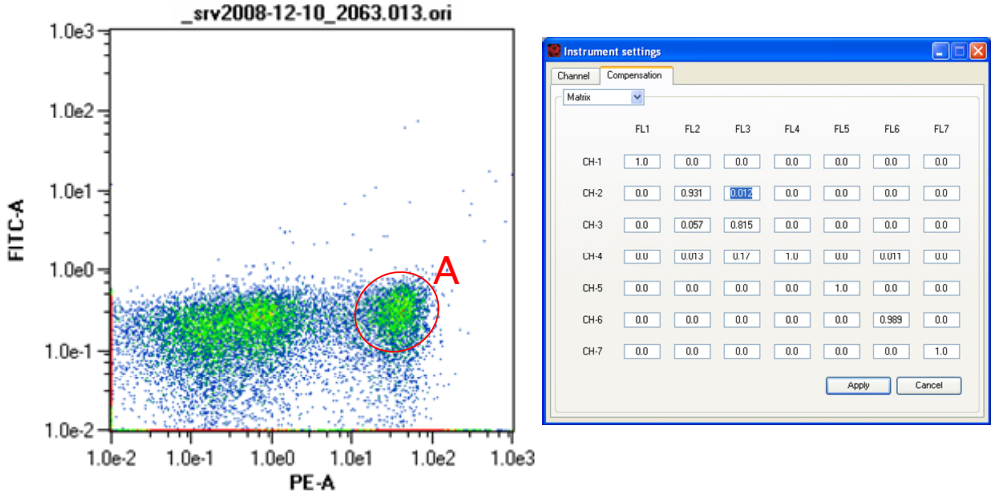


Figure 3.45 Compensated density plot with corresponding compensation matrix. Population A (bright, PE-stained cells) is no longer detected as a relatively strong fluorescence signal in the FITC channel (CH2) and has therefore been successfully compensated.

3.7.2 7-color compensation

In combination with the grouping function it is possible to perform auto-compensation with 7 stains (fluorochromes).

To perform 7-color compensation:

Prepare single-stained cells

1) Determine the cell number of a PBMC sample. Centrifuge cells (300xg for 10 minutes). Aspirate supernatant and resuspend cells in sample buffer at a concentration of $1x10^7$ cells per 100 µL buffer. Aliquot to 9 tubes.

2) Add an appropriate fluorochrome-conjugated antibody at the recommended titer to a single tube. Repeat process for another six tubes using suitable fluorochrome-conjugated antibodies, i.e., a total of 7 tubes containing single-stained cells.

> **Note:** For example, the following fluorochromes may be used for single-staining cells: VioBlue, FITC, PE, PE-Cy5, PE-Cy7, APC, APC-Cy7.

3) Leave one tube as an unstained (blank). Add propidium iodide (PI) to the final tube at the recommended concentration (see corresponding datasheet).

4) Mix well and incubate for 10 minutes in the dark at 4 °C.

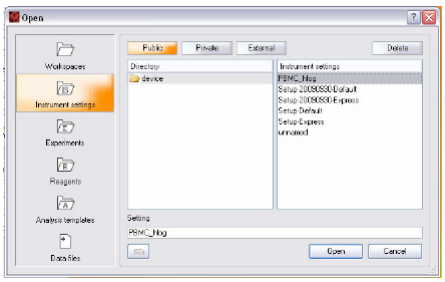
5) Wash cells by adding 1-2 mL of buffer to each tube and centrifuge at 300xg for 10 minutes. Aspirate supernatant.

6) Resuspend cell pellets to a concentration of $1x10^6$/mL.

7) Proceed to performing automated 7-color compensation on the MACSQuant Analyzer.

7-color compensation on the MACSQuant Analyzer

8) To open a saved instrument setting: click **File**, **Open** and highlight the tab **Instrument settings**. Select the appropriate file.



Note: It is not necessary to open a pre-saved instrument setting. It is also possible to use the current settings. In this case, ignore step 8.

9) Select a Chill Rack, for example, Chill Rack 5. This should be pre-cooled to 4 °C:

10) Position each of the seven tubes with single-stained cells in **columns** along the Chill Rack 5; the order should be as follows: VioBlue (A1), FITC (B1), PE (C1), PE-Cy5 (D1), PE-Cy7 (A2), APC (B2), APC-Cy7 (C2). Append the remaining two tubes containing PI stained cells (D2) and unstained cells (A3) at the end.

11) Group the samples together to form a single group as shown below.

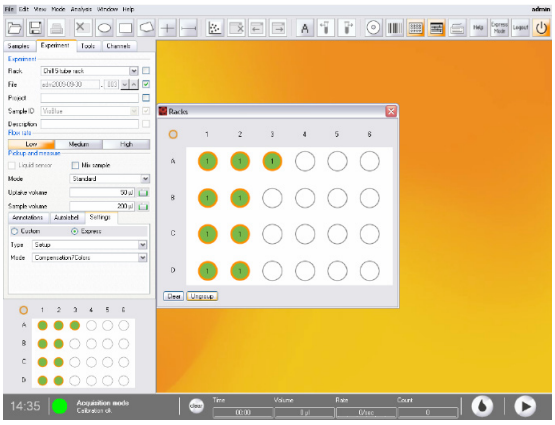


Figure 3.46 Cell samples were grouped together.

12) Click  to begin compensation.

13) The user will be prompted to draw a region of interest around a particular cell population.

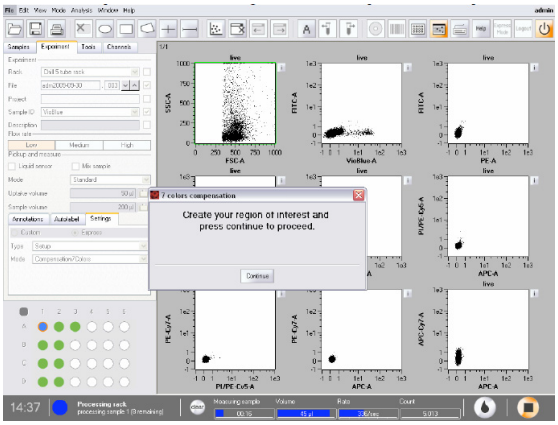


Figure 3.47 7-color compensation is underway. The user is prompted to select a region of interest before continuing.

Note: Selecting a region of interest is highly recommended. Depending on the experiment, a particular cell type or population may be selected for compensation.

14) The MACSQuant Analyzer will perform 7-color compensation on the selected region of interest,

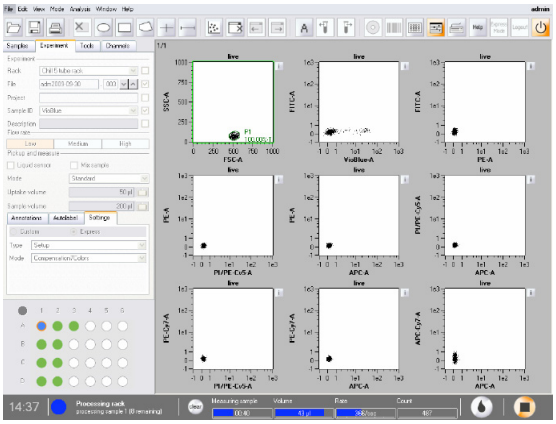


Figure 3.48 7-color compensation is underway for Region "P1"

15) Following completion of compensation the user is prompted to save the data into the current compensation bank. Click **Yes** to save current settings. Click **No** to abort the process.

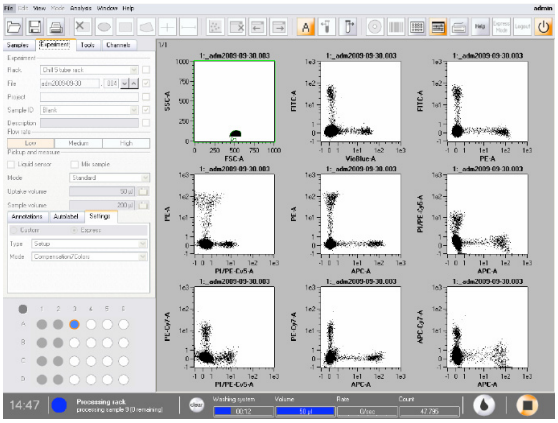


Figure 3.49 7-color compensation was successfully completed.

4 Installation of the MACSQuantify™ Software

This chapter instructs the user how to install the MACSQuantify Software onto an independent personal computer and onto the MACSQuant Analyzer.

4.1 Installing software onto a personal computer

Note: The recommended PC specifications to run the MACSQuantify Software follows:
Operating system: Microsoft® Windows® XP (SP2 is a minimum requirement although SP3 is preferred).
Memory: 1GB (minimum).

1) Insert the MACSQuantify Software DVD into the computer DVD drive. The installation program should automatically run. If this is not the case, using **Windows Explorer**, navigate to the root directory of the CD-ROM drive and execute the file **installCAP.bat**

2) At the prompt: **Do you want to install a new cap-package? [(Y)es / (A)bort]:**
Select **Y** to continue with the installation, (or **A** to abort the installation).

3) At the prompt: **Install on MACSQuant ? [(Y)es / (N)o / (A)bort]:**
Select **N** when installing the software onto a PC.
Select **A** to abort the installation.

4) At the prompt: **Do you want to keep old configurations and settings? [(Y)es / (N)o / (A)bort]:**
Select **Y** when current software configurations and settings should NOT be deleted by the new installation.
Select **N** when current software configurations and settings should be deleted by the new installation.
Select **A** to abort the installation.

5) At the prompt: **Do you want to keep all data files? [(Y)es / (N)o / (A)bort]:**

Select **Y** when saved data files should NOT be deleted by the new installation.

Select **N** when saved data files should be deleted by the new installation.

Select **A** to abort the installation.

6) The program will automatically install the software using the previously selected settings. On personal computer installations, a software shortcut icon will be created on the desktop.

4.1.1 Registering the MACSQuantify™ Software on a personal computer

Note: If your copy of MACSQuantify Software is not registered please contact Miltenyi Biotec to obtain a registration code. Visit www.macsquant.com for more information.

1) Click **Register** on the login box.

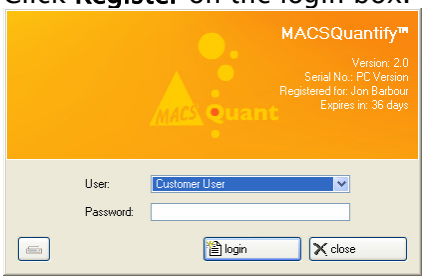


Figure 4.1 Click "Register" to register your name and registration code. The registration code is obtained from Miltenyi Biotec.

2) Enter your name and registration code as it exactly appears on the document provided by Miltenyi Biotec.

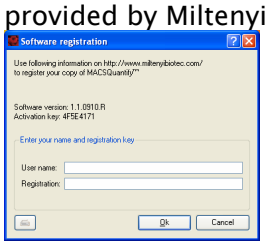


Figure 4.2 Registering MACSQuantify Software.

Note: The Name and registration code fields are case sensitive.

4.2 Updating the MACSQuantify™ Software on the MACSQuant® Analyzer

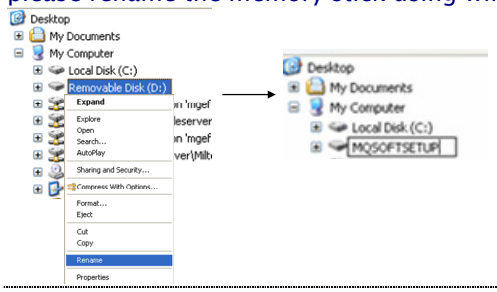
Note: The software is preloaded on MACSQuant Instrument operating system (embedded Microsoft® Windows® XP). Software updates can be easily made using a USB stick.

Note: Perform performing a software update it is highly recommended to perform a system backup.

1) Visit www.macsquant.com to download the most recent version of the MACSQuantify™ Software from your personal computer or Apple Macintosh.

2) Copy the installation program to an 'empty' memory stick i.e. ensure that no other files are already stored on the stick.

Note: The memory stick MUST be labelled as "MQSOFTSETUP". If this is not the case, please rename the memory stick using windows explorer.



3) Attach the memory stick to one of the USB ports located at the back of the MACSQuant Analyzer.

4) Login as administrator to the MACSQuant Analyzer.

5) Click **Tools** tab and **Update Software…**

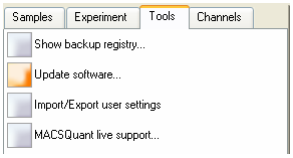


Figure 4.3 Updating MACSQuantify Software on the MACSQuant Analyzer

6) Follow the prompts to complete installation.

4.2.1 Registering the MACSQuantify™ Software on the MACSQuant® Analyzer

Note: If your copy of MACSQuantify Software is not registered please contact Miltenyi Biotec to obtain a registration code. Visit www.macsquant.com for more information.

1) Switch on the MACSQuant Analyzer.

2) Click **Register** on the opening login box.

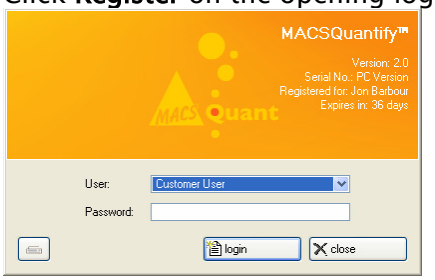


Figure 4.4 Click "Register" to register your name and registration code. The registration code is obtained from Miltenyi Biotec.

3) Enter your name and registration code as it exactly appears on the document provided by Miltenyi Biotec.

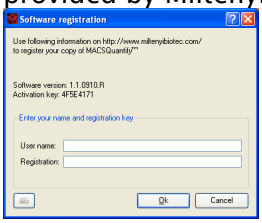


Figure 4.5 Registering MACSQuantify Software.

4) The instrument will startup and perform a system check.

5 Express mode

The **Express** mode is designed to simplify the setup, running and analysis of experiments. With only a few actions, users with minimal flow cytometry experience can perform complex flow cytometry experiments. Each user's settings are determined by the administrator at the time of the creation of the user profile. Express mode users are allowed to perform only minimal alterations to settings. To modify **Express** mode settings and/or gain access to more advanced options, the **Custom** mode must be used.

> **Note**: Sole **Express** mode users do not have permission rights to perform **Calibration** or **Compensation**. These options are restricted to Administrators in **Custom** mode and are located under the **Mode** and **Setup** drop-down list.

5.1 Quick guide to the Express mode main workspace

The **Express** mode main menu for the user "Express User (EU)" is represented below.

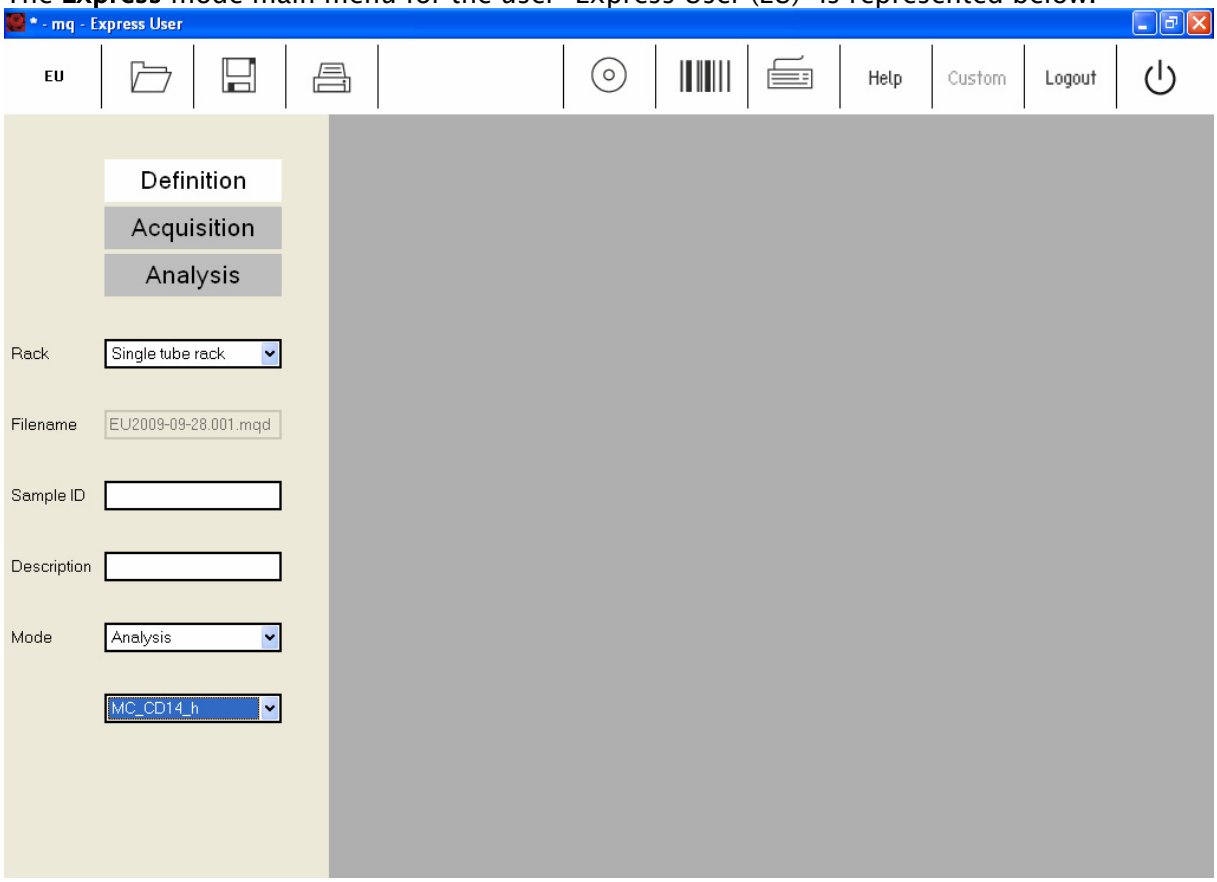


Figure 5.1 Express mode main menu

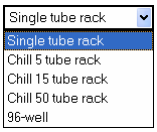
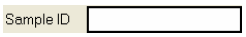
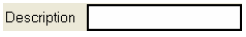
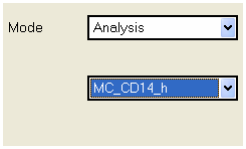
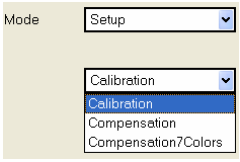
Category/icon	Description
Definition	**Definition** is used to setup an experiment, i.e., enter sample details such as name, description and required analysis mode.
Acquisition	**Acquisition** mode displays live data that is being acquired.
Analysis	**Analysis** mode is used to analyze acquired data, for example, using an analysis template.
	The Single tube rack, Chill 5, 15 or 50 tube racks and 96-well formats can be chosen using the **Rack** drop-down list.
	The filename is shown is this field (not changeable). The filename is user's initials and date, followed by file number.
	Sample ID can be entered using this text field.
	Sample description can be entered using this text field.
	Selecting **Analysis** from the **Mode** drop-down list reveals a list of analysis templates available for immediate use These templates correspond to the MACS® Control Cocktails and count programs. For more information on the MACS® Control Cocktails, please see www.miltenyibiotec.com.
	Selecting **Setup** from the **Mode** drop-down list reveals three options for instrument setup: **Calibration**, **Compensation** and **Compensation7Colors**. NOTE: Setup is only available to administrators and Custom users.
EU	Initials of user in the top left corner, in this example, "Express User" (EU).
	Folder icon to open Workspaces, Instrument Settings, Experiments, Analysis templates and/or Data files, depending on user access rights set by the administrator.
	Click to save **Workspaces**, **Instrument settings**, **Experiments** and **Analysis templates**. depending on user access rights set by the administrator-
	Print
	Backup data to DVD or initiate data transfer to USB or network location.
	Activate the 2D code (barcode) scanner.
	Activate touch screen keyboard
Help	Open help file.
Custom	Switch to Custom mode. Only available to users with Custom or Administrator rights.
Logout	Click to logout from the session.
	Main instrument control. Click to switch between Acquisition mode, Data analysis mode or Instrument off.

Table 5.1 Express interface icons with brief explanation

5.2 Login to Express mode

1) Select your user name from the dropdown list and enter the appropriate password, if required.

2) Click **login** to proceed.

Note: If the user has been registered as an Express mode user, the MACSQuantify Software will automatically log into the Express mode. If the user has been registered as a Custom mode user, the MACSQuantify Software will automatically log into the Custom mode.

Note: Please contact your administrator if there is no suitable user name and/or the password is incorrect.

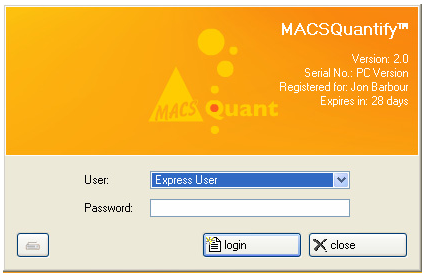


Figure 5.2 Logging-in the user "Express User" to the MACSQuantify Software in Express mode.

5.3 Switching to Express mode from Custom mode

1) In Custom mode click **Express mode** button in the top right-hand of the navigation bar.

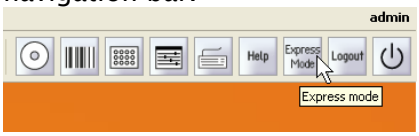
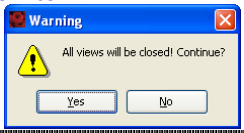


Figure 5.3 Switching to Express mode from Custom mode.

2) The MACSQuantify Software window will change to the Express mode.

Note: If windows are active in the Custom mode (e.g. analysis window), the user will be prompted to confirm this action. Click **Yes** to continue and **No** to cancel the switch.



Note: Any active work will **NOT** be transferred to the Express mode. All data or settings must be saved before switching to Express mode.

5.4 Using the touchscreen keyboard on the MACSQuant Analyzer

The touchscreen keyboard can be used to enter information into the **Sample ID** and **Description** fields. Users may find it easier to use a conventional keyboard and mouse, which may be connected to the back of the analyzer as described in section 12.1.
To activate the touchscreen keyboard perform the following:

1) Click the **Keyboard** icon,  .
2) The Keyboard popup window will appear.
3) Click the **Keyboard** icon once again to close touchscreen keyboard.

5.5 Defining an experiment

Note: All experiment and analysis templates are defined by the administrator or a Custom user. The express user may apply these settings to newly acquired data, but can not create analysis templates.

In order to perform an experiment **that the following criteria must be defined:**

5.5.1 Rack

Five different kinds of sample tube racks are available (see Table 5.2 for details). The Tube Racks Chill 5, Chill 15, Chill 50 and 96-well must be used with the MACS MiniSampler.

To select a rack configuration perform the following steps:

1) Click on **Definition** to define the experimental setup.
2) Choose the rack format using the **Rack** dropdown list. In this example, **Chill 5 tube rack** was chosen for the measurement.

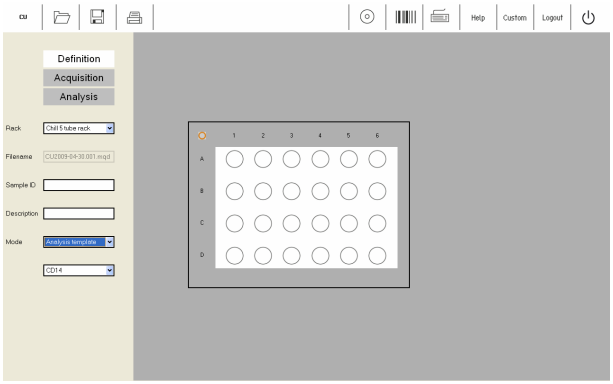


Figure 5.4 Selecting the Chill 5 tube rack for multisample labeling and cell analysis

3) For details on how to choose the correct rack format refer to Table 5.2.

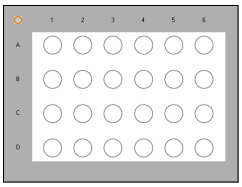
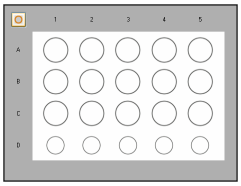
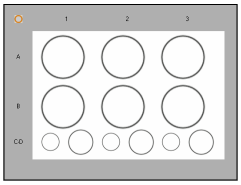
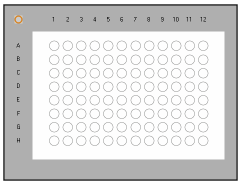
Rack type	Slots	Option on MACSQuantify Rack drop-down list	Corresponding MACSQuantify Rack Graphic
Single tube rack	1 × 5 mL	Single tube rack	Not applicable
Chill 5	24 × 5 mL	Chill 5 tube rack	
Chill 15	15 × 15 mL 5 × 5 mL	Chill 15 tube rack	
Chill 50	6 × 50 mL 3 × 15 mL 3 × 5 mL	Chill 50 tube rack	
Chill 96 rack/ 96 rack	96-well microtiter plate	96-well	

Table 5.2 Overview of the various rack types that may be used with the MACSQuant Analyzer. An appropriate rack should be used, depending on the sample number and volume.

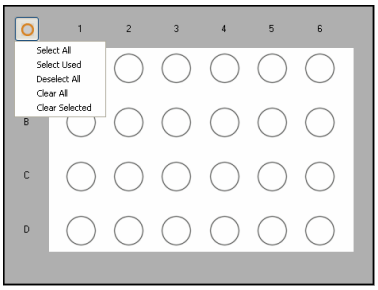
Configuring the sample rack

1) Click on a sample position using the left mouse button. This will allow you to select/deselect and activate/deactivate the sample. Refer to Table 5.3 below for a summary of the potential rack configurations for single sample positions.

User action with left mouse button or fingertip action on touch-screen	Effect	Details
None		Default open circle indicates no operation: Clear
Single click on circle/single finger-touch		Closed green circle with orange rim: Sample selected for measurement. Orange circle indicates that the sample is activated and any alterations made to the measurement strategy (e.g. labeling) will only apply to sample positions with this designation.
Double click on circle/double finger-touch		Closed green circle: Sample selected for measurement
None		Closed blue circle: Measurement in progress
None		Closed gray circle: Measurement finished
None		Closed yellow circle: Processing of sample has commenced, e.g., sample has been labeled and incubation is underway

Table 5.3 Summary of rack configurations

2) Use the right-click button to select/deselect single or multiple sample positions. The entire rack may also be selected/deselected or even cleared using the **multiple sample menu** button located at the top left-hand corner of the dialog box. Rows or columns can also be selected or deselected by clicking on the letter or number, respectively.

User action to select multiple sample positions	Effect	Details
Single right click of the multiple sample menu button.		Use this button to change the settings for all rack positions. Note: In order to set all rack positions to allow Measurement and modification of the experiment strategy (e.g. labeling): Click Select All

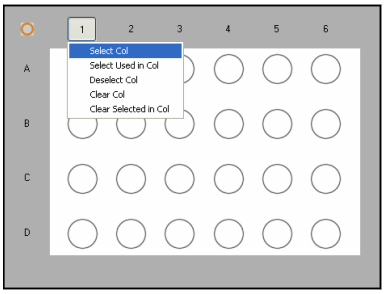
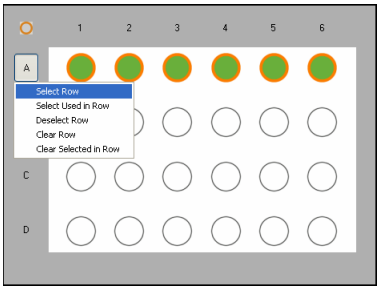
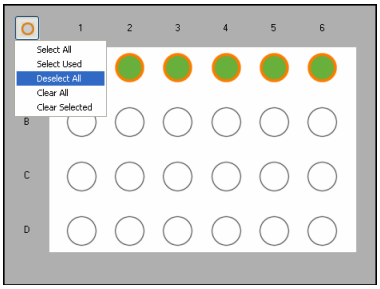
Single right click on column header		Selection/deselection of an entire sample column.
Single right click on row		Selection/deselection of an entire sample row. In this example: Row A is selected for sample labeling and measuring. Row B is selected for sample measurement only.
Single right click over a single rack position		Right click over a single rack position to completely clear this position. In this example, position A2 will be cleared.

Table 5.4 An overview of the possible configurations for rack positions.

5.5.2 Sample ID and Description

The **Sample ID** and **Description** boxes are text fields in which alphanumeric characters may be entered in order to name the sample (**Sample ID**) and provide a more extensive description (**Description**) about the sample. The **Description** field is optional.

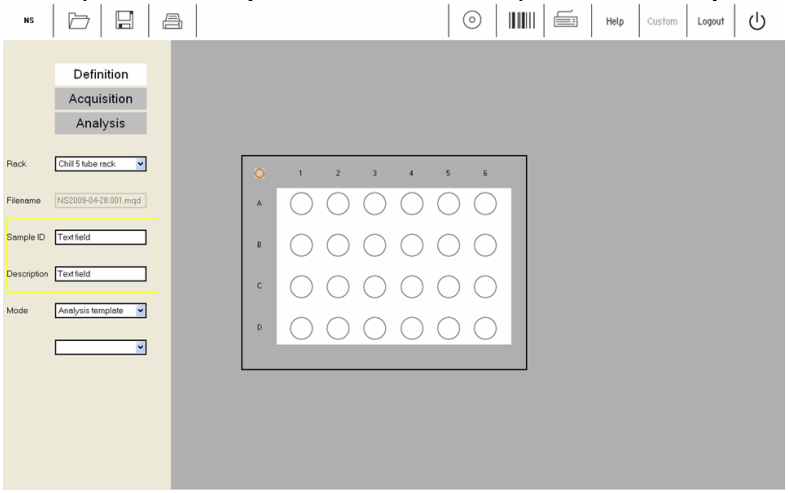


Figure 5.5 Location of the "Sample ID" and "Description" fields.

Entering Sample ID and Description text

1) Click on the Sample ID or Description text box.

2) Either the appropriate alphanumeric text using the keyboard.

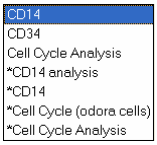
> **Note:** To use the touch-screen keyboard click on the toolbar icon,  .

3) The data is automatically stored in the field.

5.5.3 Mode

The **Mode** dropdown box has two options:

- **Analysis template**: Analysis templates simplify data analysis so that even inexperienced flow cytometry users can perform complex data analysis. Analysis templates (e.g. "gating" strategies) can be only created by administrators or custom users.
  For example, the following "gating strategies" were defined by an administrator or customer:



- **Analysis**: The **Analysis** option from the **Mode** dropdown list reveals a list of options available to perform flow cytometric cell analysis. For example, "CD14" allows for analysis of cells separated using CD14 MicroBeads i.e. application of the MACS Control CD14 Monocyte Cocktail.

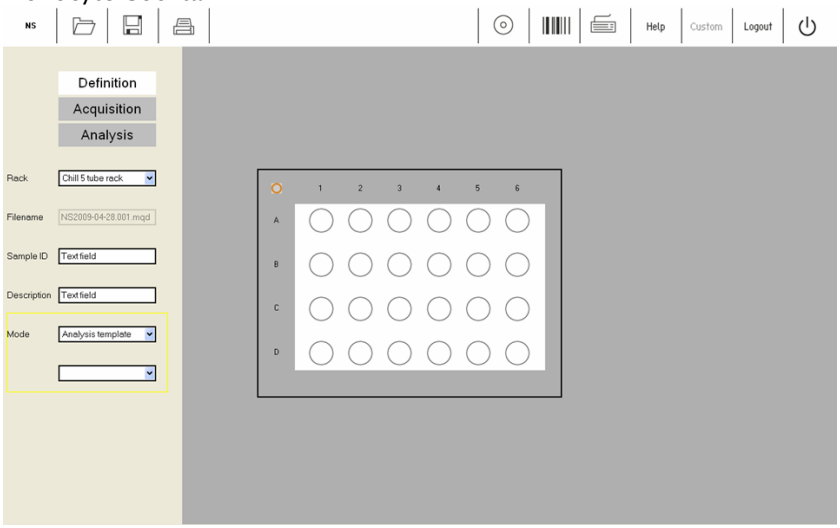
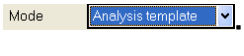


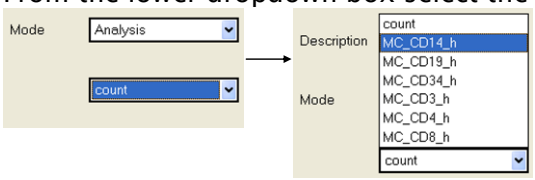
Figure 5.6 Location of the Mode dropdown box.

> **Note:** Analysis templates or Analysis options cannot be created or modified by **Express** users.

Selecting an analysis template mode

1) Select **Analysis template** from the Mode dropdown menu: 
.

2) From the lower dropdown box select the desired template:



3) Click **Start Measurement**,  .

Note: Contact your MACSQuant Analyzer administrator if the desired template is not available. Templates can only be created and managed by administrators or Custom users.

Selecting an analysis mode

1) Select **Analysis** from the Mode dropdown menu: 

.

2) From the lower dropdown box select the desired **Analysis** criterion:

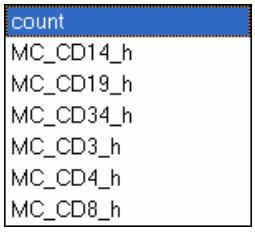
Corresponding graphic	Option	Description
	Count	To perform absolute cell counting.
	MC_CD14_h	Evaluation of MACS Cell Separations using CD14 MicroBeads, human, or the Monocyte Isolation Kit II, human (order number: 130-092-859).
	MC_CD_19_h	Evaluation of MACS Cell Separations using the B Cell Isolation Kit, human (order number: 130-092-860).
	MC_CD_34_h	Evaluation of MACS Control: MC CD34 Stem Cell Cocktail, human (order number: 130-093-427).
	MC_CD3_h	Evaluation of MACS Cell Separations using the Pan T Cell Isolation Kit II, human, or CD3 MicroBeads, human (order number: 130-092-881).
	MC_CD4_h	Evaluation of MACS Cell Separations using the CD4+ T Cell Isolation Kit II, human, or CD4 MicroBeads, human (order number 130-092-914).
	MC_CD8_h	Evaluation of MACS Cell Separations using the CD8+ T Cell Isolation Kit, human, or CD8 MicroBeads, human (order number 130-092-912).

Table 5.5 Example of options available for performing cell analysis in Express mode.

3) Click Start Measurement,  .

5.6 Working with data files in Express mode

Refer to the sections “Opening files” and “Saving files” for immediate instructions or handling these file types. If you are unfamiliar with the user interface or options associated with handling files, read the following information “Introduction to file handling”.

5.6.1 Introduction to file handling

This section describes how data files can be opened, saved, and backed-up in **Express** mode. Data files may be stored to and therefore opened from a **Public**, **Private** or **External** file location.

- Public files are located on the local hard drive of the MACSQuant Analyzer (or personal computer) and are accessible by all users.
- Private files are located on the local hard drive of the MACSQuant Analyzer (or personal computer) and are only accessible by the logged-in user account.
- External files are located on an independent file storage device which is connected to the MACSQuant Analyzer (or personal computer) via the USB port i.e. a memory stick.

The default window for saving and opening data files is composed of the following tabs:

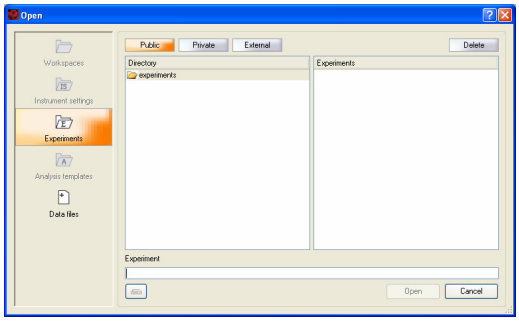
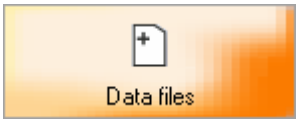


Figure 5.7 The default window for opening and saving various file types.
Note: The availability of these tab options is dependant on the user profile (Custom user, Express user or administrator) and whether data/settings are being saved or opened.

Tab option	Description
	The Workspaces tab allow users to save user an entire workspace which is composed of instrument settings, experiment and reagent definitions, and an analysis template with accompanying data.
	Instrument settings are compensation and calibration parameters for the MACSQuant Analyser. These parameters are important for data analysis and are vital to maintain standardized results over time and from instrument to instrument. The MACSQuantify Software can open and save instrument settings. These settings can be applied to acquired data and thus this useful feature allows users to perform recompensation after data acquisition.

	The **Instrument settings** may be saved but not opened in **Express** mode.
	Experiment definitions can be saved for future use. Reagent type and corresponding Reagent Rack 4 positions, sample rack type and corresponding Chill Rack sample positions, the analysis mode and sample processing definitions (e.g. labeling strategy) comprise experiment definitions.
	Reagent type and position on the reagent rack can be saved using the **Reagents** tab. The **Reagents** tab is not available in Express more.
	**Analysis templates** are predefined analysis layouts for data acquired by the MACSQuant Analyzer. The templates are created by defining a gating strategy with associated plots, histograms, tables and statistics. Administrators and Custom users can customize and save templates for reuse. Express users cannot create or modify **Analysis templates**.
	**Data files** can be saved to a **Public**, **Private** or **External** file location by all users. MACSQuant Data (MQD) is the standard file handling format, however, the MACSQuantify Software can also import Flow Cytometry Standard (FCS) file types.

5.6.2 Opening files

1) Click  to open the **Open** window.

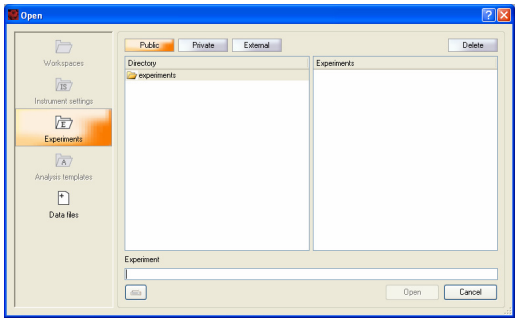


Figure 5.8 Only "Experiments" or "Data files" may by opened by Express mode users. Custom mode users and administrators are able to open Workspaces, Instrument settings and Analysis templates in custom mode.

2) Click on the **Experiment** tab or **Data file** tab to open an experiment definition or data files, respectively.

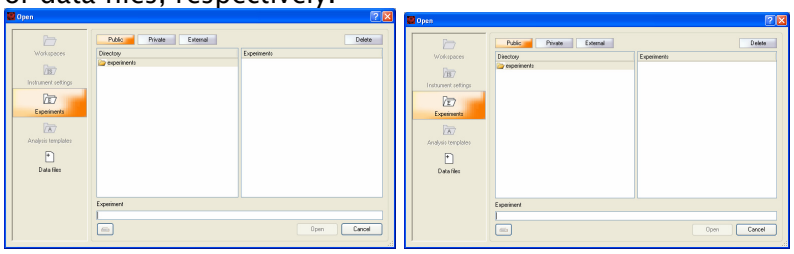


Figure 5.9 Highlight the "Experiment" (left) or "Data files" (right) tabs to open the desired file type.

To open experiment definitions:

1) Highlight the **Experiment** tab on the **Open** window.
2) Highlight the file location: **Private**, **Public** or **External**.
3) Select the file type and click **Open**, Open.

To open data files:

1) Highlight the **Data files** tab on the **Open** window.
2) Highlight the file location: **Private**, **Public** or **External**.
3) Select the file type and click **Open**, Open.

5.6.3 Saving files

1) Click  to open the **Save** window.

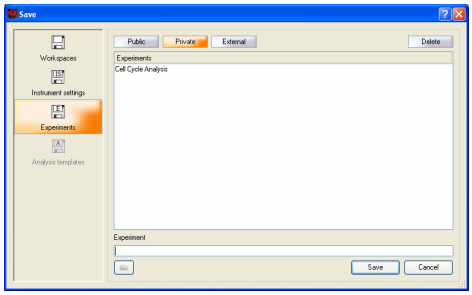


Figure 5.10 The Save window. All users are able to save experiment descriptions, instrument settings and workspaces.

2) Click on the **Experiment** tab, **Instrument settings** tab or **Workspace** tab to save the relevant file type.

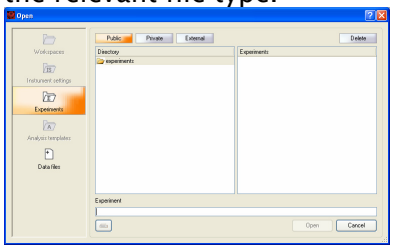


Figure 5.11 Highlight "Workspaces" (top left), "Instrument settings" (top right) or "Experiments" (bottom left) to save the desired file type.

To save a workspace:

1) Highlight the **Workspace** tab on the **Save** window.
2) Highlight the desired file location **Private**, **Public** or **External**
3) By default the workspace will be saved to the user's private folder. To save to an external drive highlight the **External** tab: External

Note: If no external media is attached to the MACSQuant Analyzer (or personal computer) USB port, the follow error will be reported:

4) Enter the filename in the **Setting** field and click **Save**, Save.

To save an experiment definition:

1) Highlight the **Experiment** tab on the **Save** window.
2) Highlight the desired file location **Private**, **Public** or **External**
3) By default the experiment definition will be saved to the user's private folder. To save to an external drive highlight the **External** tab: External

Note: If no external media is attached to the MACSQuant Analyzer (or personal computer) USB port, the following error will be reported:

4) Enter the filename in the **Experiment** field and click **Save**, Save.

5.7 Defining an experiment in Express mode: A work-through example

In the following example, three samples were placed in rack positions A1, A2 and A3 of a Chill 5 tube rack. It is intended that sample positions A1 and A2 will be analyzed using the MACS Control CD14 Monocyte Cocktail. Sample position A3 will be analyzed using the MACS Control CD4 T Cell Cocktail.

Note: Ensure that the instrument is primed and calibrated. Check that adequate reagents and buffer volumes are provided.

Ensure that the **Definition** tab is activated: Definition

1) Select **Chill 5 tube rack** from the **Rack** dropdown menu.

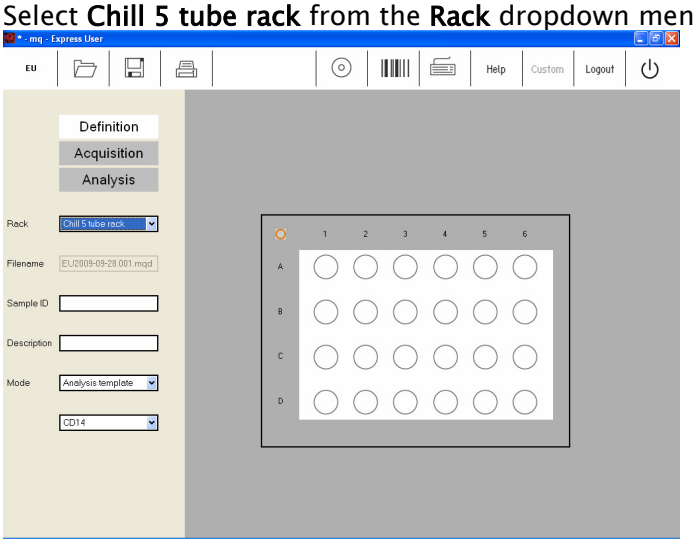


Figure 5.12 Selecting the Chill 5 tube rack

2) Left-click once on rack coordinates A1 and A2.

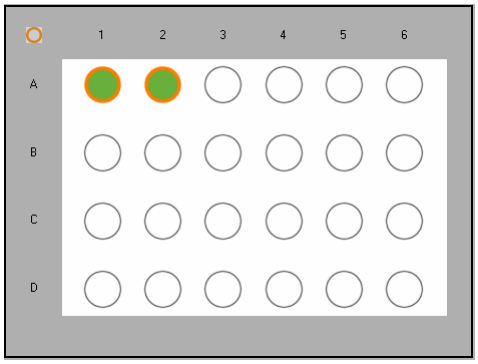


Figure 5.13 "Measure and select": The settings for sample positions A1 and A2 may be modified , e.g., a labeling strategy may be applied.

3) Select the **Analysis** Mode and **MC_CD14_h** from the lower dropdown list.

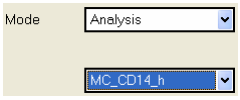


Figure 5.14 CD14 (MACS Control Cocktail) analysis is applied to rack positions A1 and A2.

4) Use the **Sample ID** and **Description** fields to enter relevant sample information.

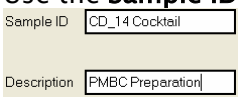


Figure 5.15 The above information is now associated with sample positions A1 and A2.

5) Select position A3.

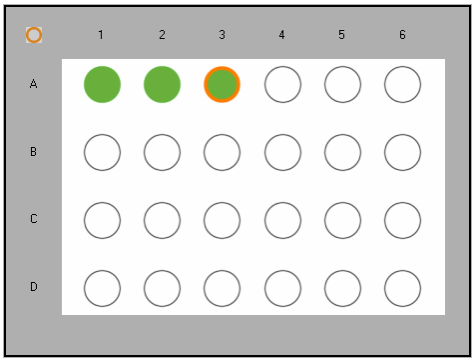


Figure 5.16 Position A3 is selected. In doing so, positions A1 and A2 are automatically deselected.

6) Select the **Analysis** Mode and **MC_CD4_h** from the lower dropdown list.

7) Use the **Sample ID** and **Description** fields to enter relevant sample information.

Note: If required, click |  | to save the experiment definitions for future use.

8) Ensure that:

The samples are correctly positioned on the reagent rack and that the MACSQuant Analyzer is provided with adequate buffer.

The waste bottle is empty.

The instrument is correctly calibrated and compensated.

9) Click **Start Measurement**,  , to start analysis.

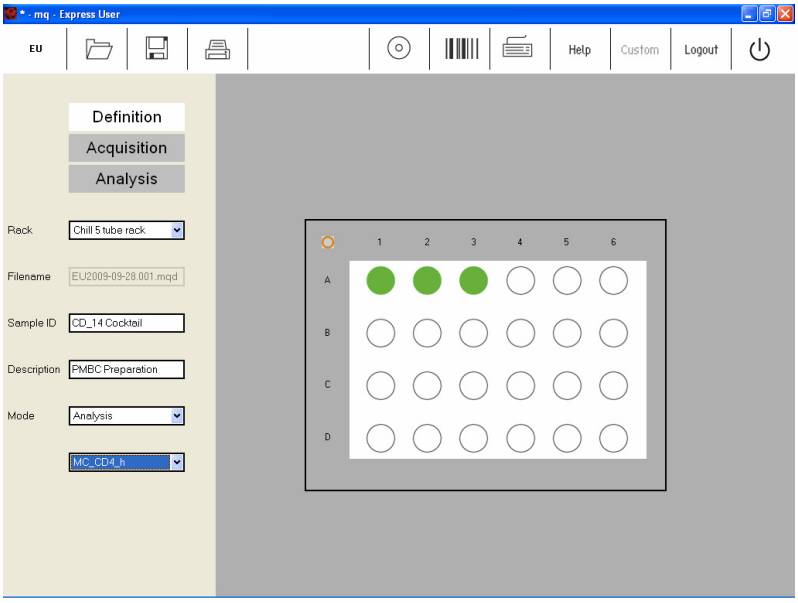


Figure 5.17 The experiment has been defined. By clicking "Start Measurement" the instrument will change to "Acquisition" mode.

Note: By saving the Experiment definition (see Step 7 above) the user can reapply the definition by clicking  , selecting the appropriate file and clicking **Open**.

10) The instrument will proceed to Acquisition mode.

11) Following data acquisition the MACSQuant Analyzer will automatically proceed to **Analysis** mode.

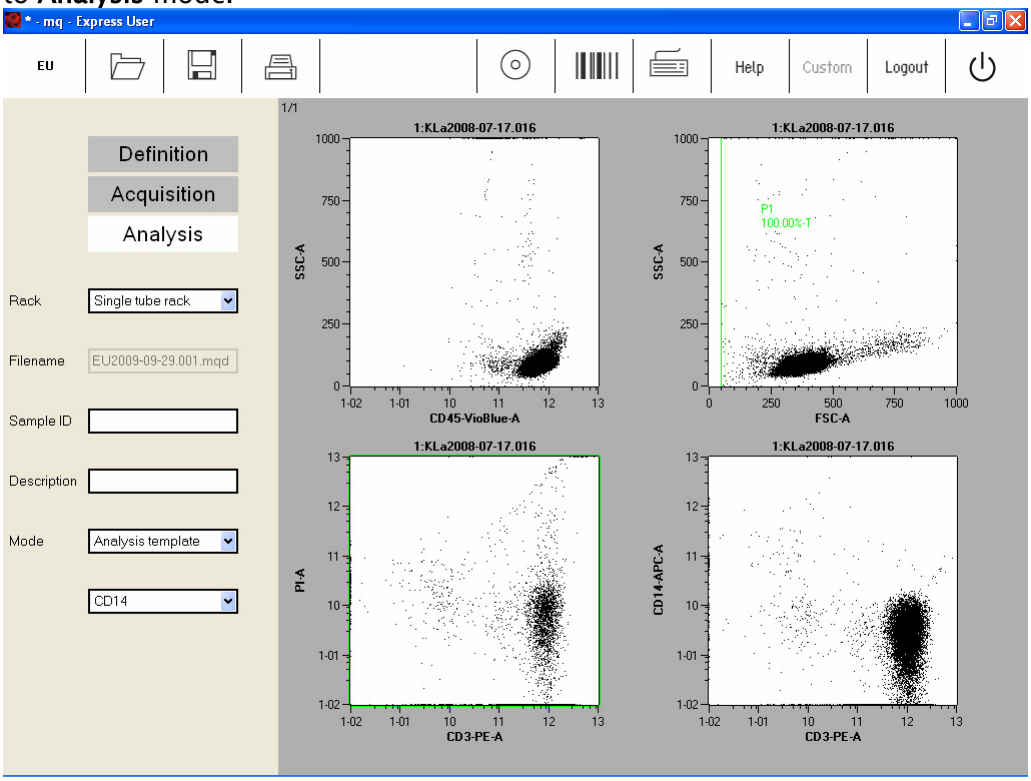


Figure 5.18 Analysis mode: CD14+ cells isolated from PMBC using MACS MicroBeads were analyzed using the MACS Control (MC) CD14 Monocyte Cocktail, human. The positive fraction is shown. A total of 1.56x10^5 viable monocytes were enriched from the original PBMC fraction, which accounted for an enrichment rate of 69.7%. The analysis template "CD14" automatically generated the "gating" strategy and associated statistics.

5.8 Reading reagents with the code reader in Express mode

The 2D code reader ("barcode reader") is used to scan reagent vials. Reagent vials are automatically recognized and logged by the MACSQuantify Software.

To scan reagents perform the following:

1) Click the activate code reader icon,  . The code reader will being blinking.

2) Present the reagent vial in front of the 2D code reader. Ensure the 2D code is facing the blinking code-reader light. The optimal reading distance is 0.5–2.5

cm from the code reader cover, tilt the vial as depicted in Figure 5.19.

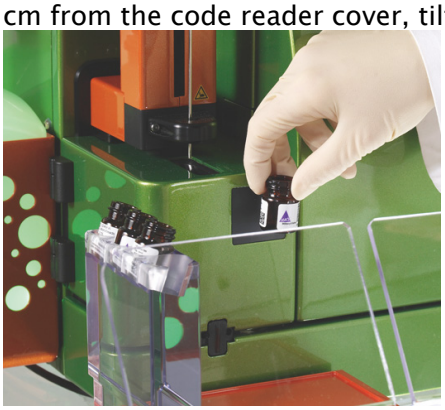
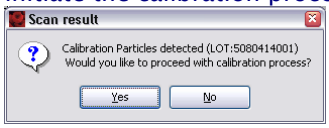


Figure 5.19 Scanning a reagent using the MACSQuant Analyzer 2D code reader.

3) Scanned reagents are reported by a MACSQuantify Software dialog box.

Note: When scanning MACSQuant Calibration Beads the instrument will prompt to initiate the calibration procedure:



Note: When scanning MACS Reagents the MACSQuantify Software will prompt the user to place the vial(s) on the MACS Reagent Rack.

Note: Contact your administrator if the code reader fails to recognize a reagent vial.

Note: Administrators and Custom Users should refer to section 6.6 for further assistance.

5.9 Printing in Express mode

The MACSQuantify Software uses installed windows printer drivers to print active workspaces.

Note: The HP Universal Print driver has been installed on the MACSQuant Analyzer and has been tested with the following printers:

Hp Laserjet – P2055d; P3005n; CP1515n; PC2025n
Hp Officejet Pro 8000

For a complete list of printers compatible with the HP Universal Print driver, please visit: www.hp.com/go/upd. Please note the only the above mentioned printers have been tested with the MACSQuant Analyzer.

Note: It is also possible to print to a network printer. Please contact your MACSQuant Analyzer administrator or Miltenyi Biotec technical support for more information.

To print active workspaces:

1) Open the desired workspace or analysis window.

2) Click |  |.

3) Select the desired printer. Click **Print**.
The printer can be networked to or directly connected to the MACSQuant Analyzer or to the PC running the MACSQuantify Software.

4) The active workspace is printed as shown below.

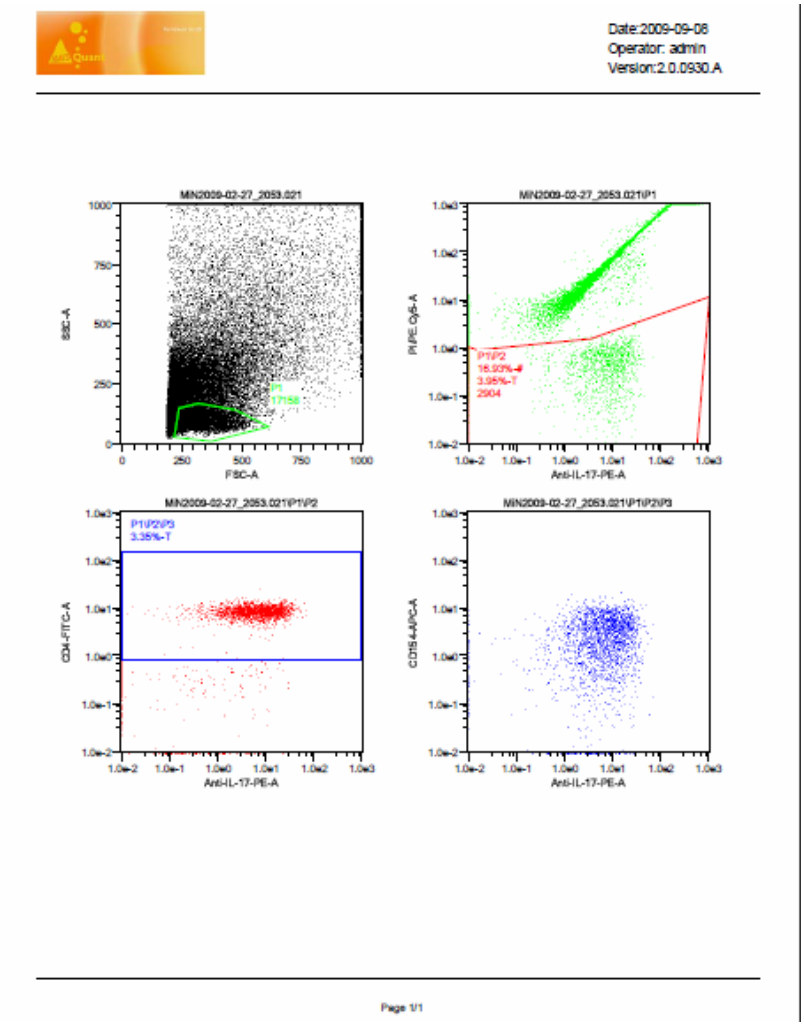


Figure 5.20 Half-size example print-out of data analyzed by the MACSQuantify Software in Express mode.

5.10 MACSQuant Analyzer data backup in Express mode

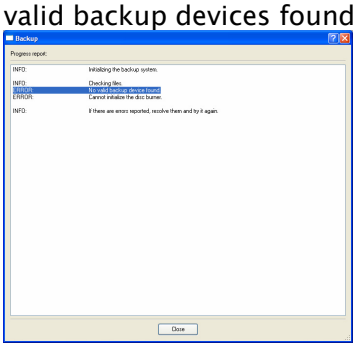
It is recommended that data is regularly backed-up to an external location. Data can backed-up to a network drive, USB memory stick or DVD. Administrators can configure data backup settings. Please contact your administrator for more information or refer to section 6.11.4 of this help guide.

> **Note:** Before performing **Backup**, ensure that the desired backup media is accessible to the MACSQuantify Software.

Backup media

The backup procedure ( ) searches for backup media in the following order:

1) A designated folder located on a local area network: this must be setup by an administrator with assistance from Miltenyi Biotec technical support.
2) A memory stick attached to the USB port on the MACSQuant Analyzer.
3) A rewritable DVD.
4) If none of the above are found, the MACSQuantify Software reports an error: No valid backup devices found.



5.10.1 To perform a backup to a rewritable DVD

1) Ensure no USB stick is installed and that no network drive has been defined as the default location for backup files. Please contact your administrator for further advice.
2) Insert a rewritable DVD into the MACSQuant Analyzer DVD drive. Only DVD-R or DVD-RW media may be used. DVD+RW and CD media types are not currently supported.
3) Wait for 10-20 seconds after inserting the DVD into the drive.
4) Click the backup icon located on the top menu bar, .
5) The files will be written to DVD.

Note: Depending on the amount of data, the backup procedure may take several minutes. When the progress bar displays 100% the MACSQuantify Software will verify the data once again; this may take a few minutes to complete.

Note: At this stage data will NOT be deleted from the MACSQuant Analyzer, the data is only copied to DVD.

6) Insert the backup DVD into the destination DVD-drive of an independent personal computer on which the MACSQuantify Software is preinstalled. This computer can be used for data analysis.

7) Start and login to MACSQuantify Software on the personal computer.

8) Click restore  .

Note: MACSQuant Analyzer data will be **copied** to the local drive of the personal computer. After a successful data transfer, the copied data will be "marked" as successfully copied on the DVD.

Note: When performing a future data backup on the MACSQuant Analyzer, ensure that this backup DVD is used.

Performing subsequent MACSQuant Analyzer backup

9) Insert the **designated** MACSQuant Analyzer backup DVD.

10) Click backup,  .

Note: The MACSQuant Analyser software (MACSQuantify) will identify data 'marked' on the DVD as successfully transferred to another computer. This corresponding data will be deleted off the MACSQuant Analyzer hard-drive and DVD before continuing with the backup procedure.

11) After backup is finished, remove the DVD from the drive.

12) Transfer the data to an independent personal computer as described above.

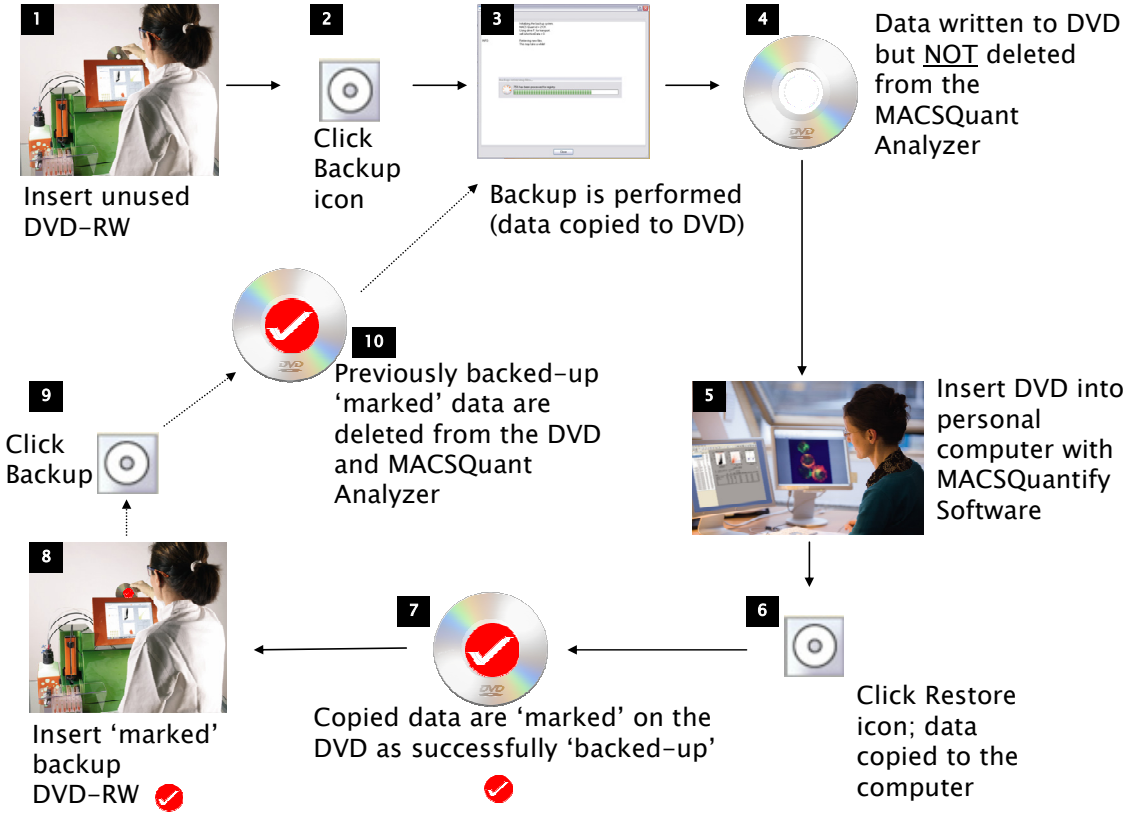


Figure 5.21 Schematic of the MACSQuantify Software/ MACSQuant Analyzer DVD backup procedure.

5.10.2 To perform backup to a USB memory stick

1) Ensure that no network drive has been defined as the default location for backup files. Please contact your administrator for further advice.

2) Insert a memory stick into the MACSQuant Analyzer USB port or USB port a personal computer. Wait a few seconds.

3) Click |  |.

4) The files will be automatically written to the USB memory stick.

Restoring files from a USB memory stick to a personal computer

5) Start MACSQuantify Software and login to a user account.

6) Insert the memory stick into a USB port of a personal computer with MACSQuantify Software installed.

7) Click **File** and **Import...**

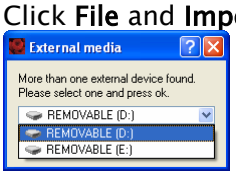
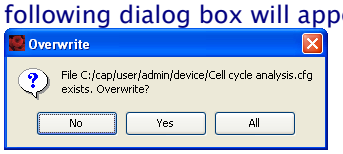


Figure 5.22 If more than one external USB is attached you will be prompted to selected the correct external device.

8) Use the dialog box to select the type of file for import e.g. Workspace, Instrument settings, Data files etc.

> **Note:** If a copy of the imported file already exists on the personal computer, the following dialog box will appear:
>
> 
>
>
> To overwrite a single file click **Yes**. To overwrite all files for import, click **All**. **No** aborts the procedure.

9) Highlight the file(s) and click **Import**.

> **Note:** The imported files are copied to MACSQuantify Software. It is necessary to delete files off the memory stick using windows explorer.
>
> **Note:** It is of course also possible to simply move files from the memory stick to a personal computer using windows explorer.

5.10.3 To perform backup to network drive

1) Please contact your administrator if a network drive has not been configured for backup.

> **Note:** If a network drive is not configured, the MACSQuant Analyzer software (MACSQuantify) will search for USB and DVD backup media instead.

2) Click  .

3) The files will be automatically written to the network drive.

5.11 Logging out from Express mode

1) Click the **Logout** icon, 

.

2) If prompted to continue, click **OK.**
The software will return to the login menu.

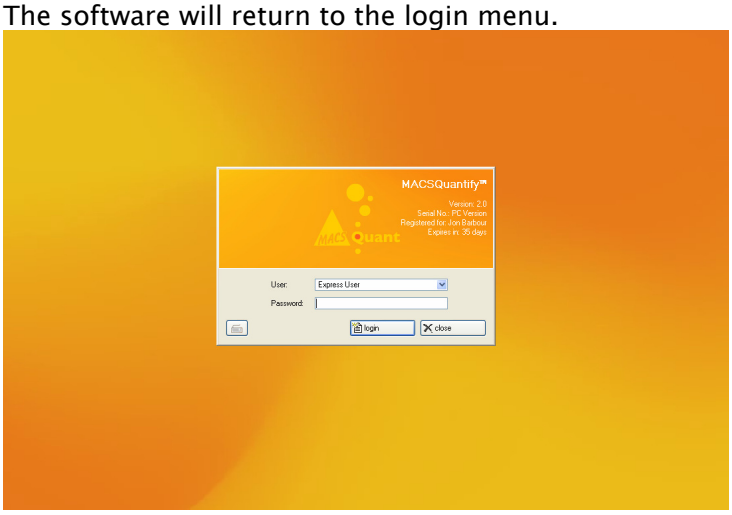


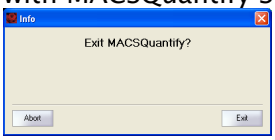
Figure 5.23 Login menu.

5.12 How to close the MACSQuantify Software

Note: This information is only applicable to personal computer users of the MACSQuantify Software. Please refer to the chapter "How to shutdown the MACSQuant Analyzer" for instructions on how the instrument may be cleaned and shutdown.

1) Click the **Shutdown/close software** icon,  .

2) Click **Exit** to continue closing the software. Click **Abort** to continue working with MACSQuantify Software.



6 Custom mode

The **Custom** mode is designed for advanced flow cytometry users. Administrators and Customer users can use the Custom mode interface to create customized experiments ranging from sample autolabeling and uptake, through data acquisition, gating and data analysis, to the generation of print-ready results. Custom users and administrators have advanced access to MACSQuant Analyzer instrument and software settings.

Administrators have additional permissions concerning setting user permissions and the management of Express and Custom mode users. Both administrator and Custom user features are discussed throughout this chapter.

6.1 Custom mode quick reference guide

The quick reference guide provides and overview to the icons and layout of the MACSQuantify Software in Custom mode.

6.1.1 Quick guide to the top menu bar icons

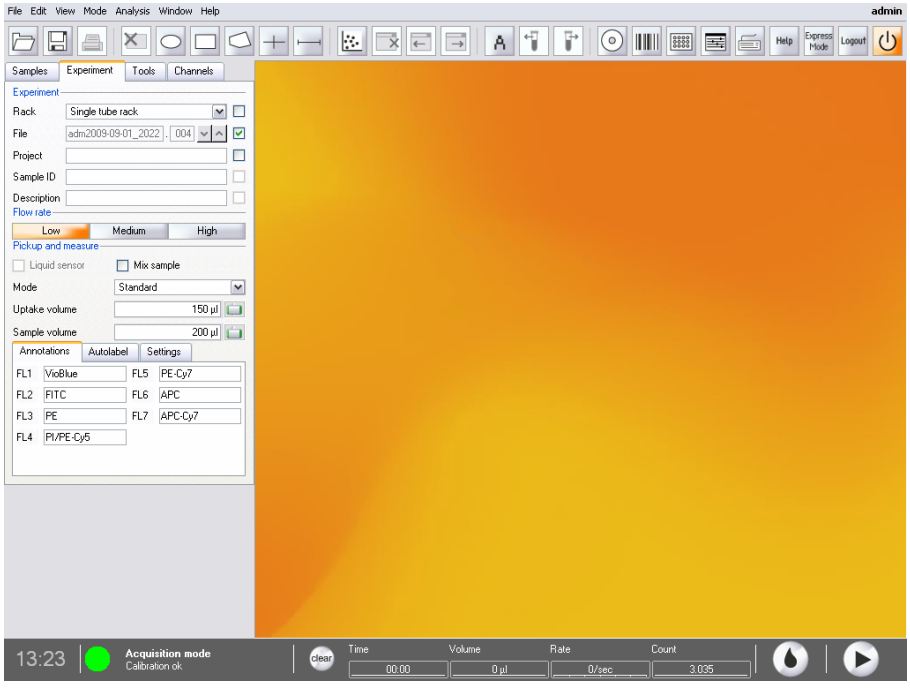


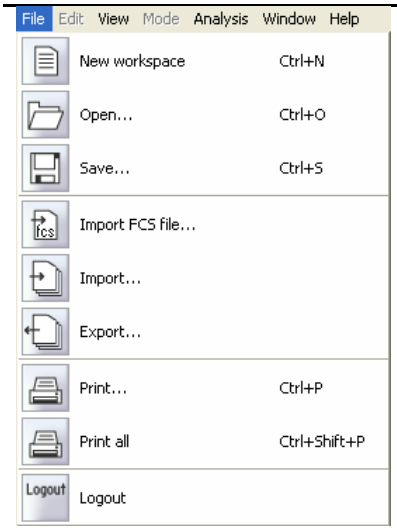
Figure 6.1 Screen shot of the custom mode main screen using the “admin” account

Icon	Description	Icon	Description
	Folder icon to open Workspaces, Instrument Settings, Experiments, Analysis templates and/or Data files, depending on user access rights set by the administrator.		Activate the Analysis template tool
	Click to save Workspaces, Instrument settings and Experiments, depending on user access rights set by the administrator		Scroll through samples listed in the samples window
	Print		Backup or transport data.
	Delete a region that was created in a dot plot or histogram.		Activate the reagent barcode scanner.
	Draw a region in a dot plot, i.e., to define an area of interest. Ellipse, rectangular and polygonal regions can be drawn.		Open the rack dialog box.
			Open the instrument settings dialog box
			Activate touch screen keyboard
	Draw a quadrant in a dot plot.		Open help file and manual.
	Draw an interval in a histogram.		Switch to Express mode.
	Open a new analysis window.		Logout user from session
	Close analysis window.		Main instrument control. Click to switch between Acquisition mode or Data analysis mode. The instrument may also be switched-off using this button.
	Scroll through open analysis windows in a reverse and forward direction.		Name of user in the top right corner, in this example, the administrator (admin) is logged-in.

Table 6.1 Quick guide to the MACSQuantify Software top menu bar icons.

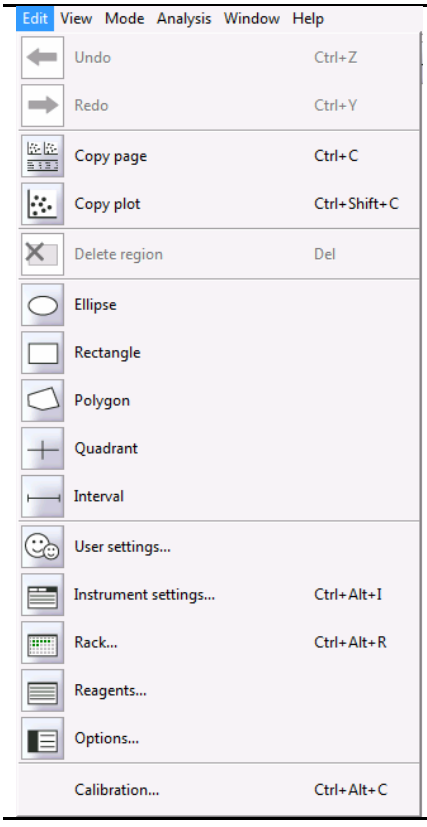
6.1.2 Quick guide to the MACSQuantify Software menus

File



Command	Description
New workspace	Create a new workspace. The user will be prompted to save any changes to the workspace before this action is performed.
Open…	Open Workspaces, Instrument Settings, Experiments, Analysis templates and/or Data files, depending on the user access rights set by the administrator.
Save…	Click to save Workspaces, Instrument settings, Experiments, Reagents and Analysis templates, depending on user access rights set by the administrator.
Import FCS file	Import data files in the FCS file compression format.
Import…	Import data files from an external media source, e.g., external hard drive
Export…	Export data files to external media, e.g. external hard drive
Print…	Print a selected area.
Print all	Print the entire workspace.
Logout	Logout of the current session

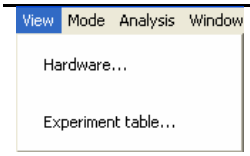
Edit



Command	Description
Undo/ Redo	Undo or redo the most recently performed action, e.g., undo – create region.
Copy page	Copy the entire analysis window to the clipboard. All dot plots and tables are copied.
Copy plot	Copy a single selected plot/histogram highlighted in green.
Delete region	Delete a region that was created in a dot plot..
Ellipse, Rectangle, Polygon, Quadrant, Interval	Create the afore mentioned geometric shape in a plot.
User settings…	Create and/or modify user account settings. **Administrator only.**
Instrument settings…	Modify the instrument settings comprising Channel, Compensation and Custom.
Rack…	To edit the sample rack settings.

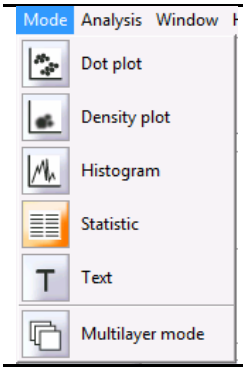
Reagents	To open the Reagents dialog box and modify reagent settings
Options…	To modify User, Experiment, Instrument and Software options. Only available as administrator.
Calibration…	To view and/or modify instrument calibration settings.

View



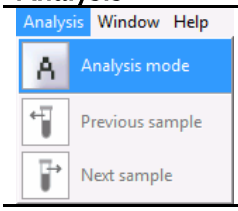
Command	Description
Hardware	To view the hardware settings comprising Fludics, Sample uptake unit, Lasers and detectors, Camera and System settings.
Experiment table	Provides a tabulated overview of experiment details: **Acquisition**, **Annotations**, **Autolabel** and **Settings**.

Mode



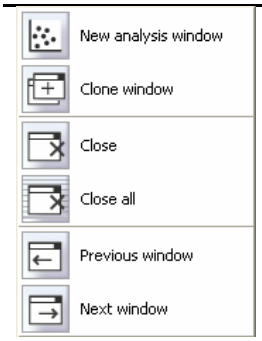
Command	Description
Dot plot, Density plot, Histogram, Statistic, Text	Click icon to change the presented data format into another format, e.g., from a dot plot into a histogram.
Multilayer mode.	View data in a multilayer format

Analysis



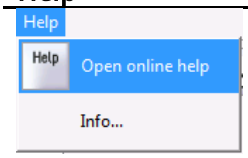
Command	Description
Analysis mode	Activate Analysis template tool.
Previous sample/ Next sample	Scroll through analysis windows in a reverse and forward direction.

Window



Command	Description
New analysis window	Open a new analysis window using predefined templates.
Clone window	An exact clone/copy of the analysis window is made. This includes gating strategies and open data files.
Close/Close all	Close a selected analysis window/close all analysis windows.
Previous window/ Next window	Sequentially scroll through analysis windows, i.e., previous and next analysis window.

Help



Command	Description
Help	Open the preinstalled help file.
Info…	Information about current software version.

6.2 User administration

6.2.1 Creating a new user

In order to optimize the unique user management system of the MACSQuant Software it is recommended to create an individual user account for each user.

1) Select **Edit** in the Windows pull-down menu and **User settings**...

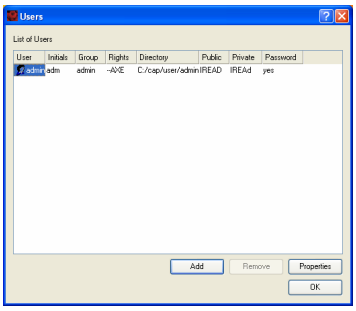


Figure 6.2 The users dialog box.

2) Click **Add** to create a new user.

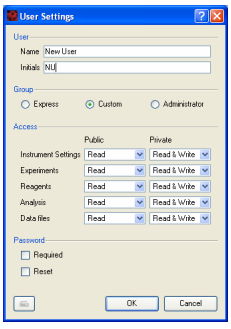


Figure 6.3 Creating a new user account

3) Enter the **Name**, **Initials** and associated access settings of the new user.

Category	Sub-category	Description
User		
	Name	Enter the user identity in this field.
	Initials	Enter the respective initials.
Group		
	Express	Check radio button to setup the account for Express mode access only.
	Custom	Check radio button to setup the account for Custom mode and Express mode access.
	Administrator	Check radio button to setup the account as an administrator.
Access		
	Instrument settings	Using the drop-down list, set the user access for each of the following criteria: None: User access is unavailable. Read: User access is restricted to read files only. Read & Write: Full user access is available, i.e., read and write data to this folder. These options are available for Public and Private accounts. Note: the data files can only be stored in one location, either Private or Public.
	Experiments	
	Reagents	
	Analysis	
	Data files	
Password		
	Required	Activate checkbox to ensure password restricted access to the account.
	Reset	Activate checkbox to reset an associated password. The user will be prompted to enter a new password at the next log in attempt.

Table 6.2 Creating a new user: setting user properties

4) Click **OK** to save the new user settings.

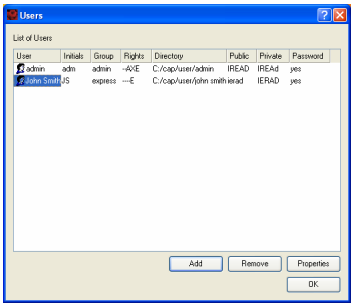


Figure 6.4 An Express password protected user account was created for John Smith (JS).

5) To modify or delete the user account, click **Properties** or **Remove**, respectively.

Note: If the user is set as an **Express** mode user, the user will be automatically logged into the Express mode. If the user is set as a **Custom** mode user, the user will be automatically logged into the Custom mode window. This same applies to administrators.

6.3 Getting started in the custom mode

This section is intended to provide the user a quick overview of actions required for a quick-start of the MACSQuant Analyzer in the custom mode. Some familiarity with the MACSQuantify Software is assumed.

6.3.1 Turn on the instrument

Switch on the MACSQuant Analyzer by pressing the touchscreen monitor (while it is in standby mode). The MACSQuant Analyzer will automatically check and initialize the system, following which the log in screen will be displayed.

6.3.2 Login as administrator or custom user

1) Switch on the analyzer.

2) At startup a dialog box will appear prompting you to select your username and password. The administrator (**admin**) account should be used for the first log in attempt.

Figure 6.5 Logging in for the first time as an administrator. A dialog box prompts the user to confirm the password.

Note: Custom users must only select their login name from the "User:" dropdown list. No further action is required.

3) Select user account (admin) and enter a new password. Select **log in.**

4) After a successful log in, the software will automatically prepare the instrument for analysis. The status of this startup procedure is indicated by the **System setup** dialog box. After a few seconds the main screen will appear in **Data analysis mode**.

6.3.3 Activate the touchscreen keyboard on the MACSQuant Analyzer

The touchscreen keyboard can be used to enter information into the **Sample ID** and **Description** fields. Users may find it easier to use a conventional keyboard and mouse, which may be connected to the back of the analyzer as described in section 12.1.
To activate the touchscreen keyboard perform the following:

1) Click the **Keyboard** icon,  .
2) The Keyboard popup window will appear.
3) Click the **Keyboard** icon once again to close touchscreen keyboard.

6.3.4 Check the fluid levels

Check that sufficient running buffer and washing solution are present in the containers for the measurement (minimum 150 mL of each). Replace any of the solutions whose levels are low. Also, check that the waste container is empty. Please note the bottles are only illuminated after the system has been placed into acquisition mode.

Note: Check fluid levels before each application!

If any buffers or solutions need to be changed:

1) Press the Main Instrument Control icon () to set the instrument into Acquisition mode.
This will initiate the priming of all of the fluidics, turn on the illumination LEDs for the four buffer bottle and turn on the lasers. (Each time you select the Main Instrument Control, a dialog box will appear providing two of three options,

Analysis mode, Acquisition mode or Instrument off).

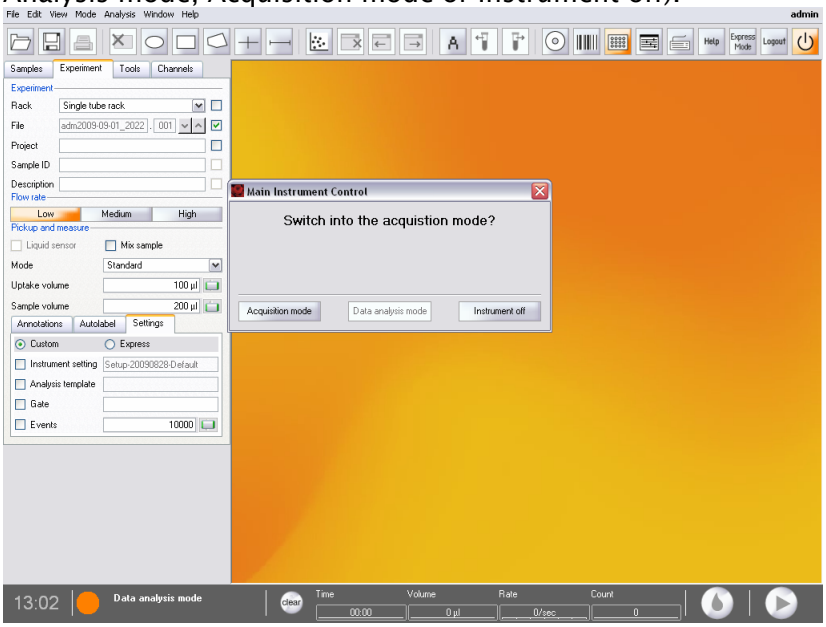


Figure 6.6 Switching the instrument into acquisition mode.

2) After the instrument has been primed and all system checks have been performed successfully, the instrument status and LEDs will display green (see section 6.3.5). If the system status is red, act in accordance with the error message. The system status will prompt you if the instrument needs to be calibrated and how many days since the last calibration.

3) If the LEDs are red or if you suspect one of the solutions is too low, you can replace the appropriate solution.

4) To replace a solution place the closed bottle into the orange solution basket prior to exchanging it. Remove the cap, and attach the bottle closure and sensor to the new bottle.

5) To empty the waste container, remove the closure while the container is still in the holder, close it with a cap. Then remove the closed container. Replace the bottle with an empty waste bottle and attach the bottle closure and sensor to the new waste bottle.

Note: Handle the full liquid waste bottle with extreme caution and dispose of as recommended by your local authority.

Note: It is recommended to add 100 mL of a MACS Bleach solution to the bottom of the waste container.

Note: Check that all connections are securely fastened and that no tubing is tangled.

6.3.5 Check the instrument status

The instrument status can be monitored using the status bar and illuminated bottles.

In order to start experiments the MACSQuant Analyzer should report the status Calibration Ok: 

. The following procedures must be completed before performing experiments on the MACSQuant Analyzer:

- Instrument hardware must be correctly installed and calibrated (see section 3.5).
- Instrument settings must be correctly calibrated and compensated (see section 3.7).

A more comprehensive explanation on monitoring the instrument status is given below.

Checking the instrument status using the status bar:
The instrument status is reported by the status bar using text and a corresponding color code.

Orange: MACSQuant Analyzer in **Data analysis mode**—the instrument can only analyze data. The instrument must be placed in acquisition mode to perform a measurement.

Figure 6.7 Instrument is in Data analysis mode

Green: MACSQuant in **Acquisition mode**—calibration was successfully performed one day ago (time in days since the last calibration is indicated).

Figure 6.8 Instrument is in Acquisition mode

Yellow: Cleaning and priming of MACSQuant Analyzer—instrument is not available for measurement, cleaning in progress.

Figure 6.9 Instrument is in the process of being cleaned

Grey: Instrument is being initialized – instrument is not available

Figure 6.10 Instrument is unavailable

Blue: MACSQuant® Analyzer is processing a sample – Instrument is currently processing a sample

Figure 6.11 Sample processing in progress

> **Note:** Upon completion of the initialization process, the MACSQuant Analyzer is in the **Data analysis mode** until the instrument is placed in Acquisition mode.

Checking the instrument status using the bottle LEDs:
The MACSQuant® Analyzer is equipped with light-emitting diodes (LEDs) which illuminate each bottle to indicate the status of the instrument in Acquisition mode:

Green bottle light: The instrument is ready to measure, liquid levels are sufficient, and the instrument is primed.

> **Note:** Please note that the lasers can take up to 30 minutes to warm-up after performing the initial instrument priming.

Purple bottle light: The instrument is measuring, liquid levels are sufficient. Blue light is indicative of normal instrument function during sample processing, or that the instrument is busy.

Red bottle light: Liquid level error/general instrument error. Red light indicates that the liquid levels are too low in a particular bottle or that the waste needs to be removed. The bottle with the blinking red light will indicate which bottle needs to be tended to. Additionally, a message on the system status in the lower left corner will refill that a bottle change is needed. A bottle can be replaced even during a measurement, although replacing the waste is best when the instrument is not processing.

Yellow bottle light: Sensor error. Please ensure that the sensor is correctly attached to the bottle.

> **Note:**If no LED is illuminating the fluid containers then the instrument is in the Data Analysis mode and the lasers are not on.

6.3.6 Define an experiment in Custom mode

> **Note:** Refer to section 6.1 for an overview of the MACSQuantify Software custom mode interface.

Before setting up an experiment the following questions should be addressed:

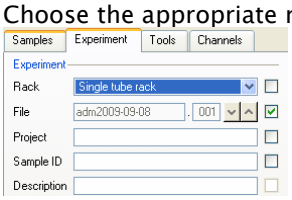
1) How many samples will be analyzed and what is the sample volume?

   - Single samples are processed using the Single Tube Sample Rack.
   - Multiple samples (up to 96) are processed by using the MACS MiniSampler in combination one of the following Cooling Tube Racks:

Rack type	Slots
Chill 5	24 × 5 mL
Chill 15	15 × 15 mL 5 × 5 mL
Chill 50	6 × 50 mL 3 × 15 mL 3 × 5 mL
Chill 96 rack/ 96 rack	96-well microtiter plate

Note: Chill Racks should be pre-cooled for 3-4 hours. Do not chill below 0 °C.

- Choose the appropriate rack and configure it menu accordingly:



Note: Refer to section 6.8 for information concerning use of the MACS MiniSampler and configuration of sample racks.

- Multiple samples (up to 96) are processed by using the MACS MiniSampler in combination one of the following Cooling Tube Racks:

2) Will autolabeling be performed or is sample analysis only required?

- Yes, autolabeling is required.
  Up to four MACS Reagents can be placed on the MACS Reagent Rack 4 for:
  1. Magnetic autolabeling of rare cell populations for subsequent pre-enrichment and analysis.
  2. Fluorochrome autolabeling of specific cell populations.
- No, autolabeling is not required.
  If samples are manually labeled no MACS Reagent Rack is required. For manual labeling follow the labeling instructions in the corresponding datasheet.

3) Are rare cells being analyzed?

- Yes, rare cell analysis is required.
  1. Rare cells can be automatically magnetically labeled and enriched by the MACS Cell Enrichment Unit. Depending on the selected analysis mode, the enriched and non-enriched fractions can be subsequently analyzed by flow cytometry. A MACS Column must be installed for pre-enrichment.

	Enrich.Measure.Ori.Neg.Pos	EnrichS.Measure.Ori.Neg.Pos	Enrich.Measure.Pos	EnrichS.Measure.Pos
Details of sample processing and analysis	Target cell population is enriched. The following cell fractions are analyzed by flow cytometry: 1. Original cell fraction ("ori"). 2. Enriched target cells ("enrich") fraction. 3. Remaining non-target cells or negative fraction ("neg").	Target cell population is enriched at a slower rate. The following cell fractions are analyzed by flow cytometry: 1. Original cell fraction ("ori"). 2. Enriched target cells ("enrich") fraction. 3. Remaining non-target cells or negative fraction ("neg").	Target cell population is enriched. The following cell fractions are analyzed by flow cytometry: 1. Enriched target cells ("enrich") fraction.	Target cell population is enriched at a slower rate. The following cell fractions are analyzed by flow cytometry: 1. Enriched target cells ("enrich") fraction.
Cell separation rate	0.5 mL/min	0.5 mL/min	0.5 mL/min	0.5 mL/min
Cell washing rate	2 mL/min	1mL/min	2 mL/min	1 mL/min
Volume of wash buffer used	3.00 mL	4.66 mL	3.00 mL	4.66 mL
Rate of elution	50 mL/min	37.5 mL/min	50 mL/min	37.5 mL/min
Elution quantity	450 µL	450 µL	450 µL	450 µL

Table 6.3 Specifications for sample pre-enrichment using the MACS Cell Enrichment Unit in combination with the MACSQuant Column.

2. MACS Control Antibody Cocktails are available for automated and reliable rare cell analysis. See http://www.miltenyibiotec.com/en/NN_662_MACS_Control_Antibody_Cocktails.aspx for a list of available products.

- No, rare cell analysis is not required.

3. The **Standard** or **Fast** modes do not involve sample pre-enrichment.

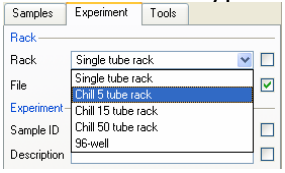
Having addressed the above questions the experiments can be easily defined using the **Experiment** tab.

Define an experiment as follows:

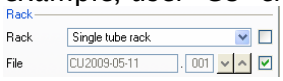
Rack

1) Click the **Experiment** tab.

2) Select the rack type using the **Rack** dropdown list.



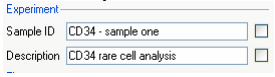
3) Optional: Change the filename.
The filename is automatically created by the MACSQuantify Software using the following nomenclature: <**User initials**><**Date** (YYYY-MM-DD)>. In this example, user "CU" created a file on 11. May, 2009.



Note: To change the filename deactivate the **File** checkbox and enter a filename into the File field.

Experiment

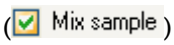
4) Enter alphanumeric text for the **Sample ID** and **Description**, for example:



Flow rate

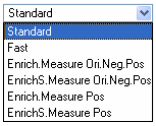
5) Select a flow rate: **Low**, **Medium** or **High**.

Pickup and measure

6) Optional: Click the **Mix sample** checkbox (

) to premix the sample before sample pickup, data acquisition and analysis.

7) Select an analysis mode from the **Mode** dropdown list:



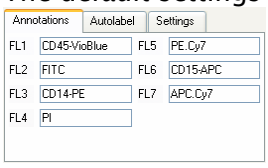
See Table 6.3 for details about each option.

8) Enter the **Uptake volume** and **Sample volume**.

A maximum sample volume of 5 mL and a maximum uptake volume of 450 µL can be entered.

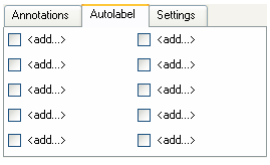
Annotations tab

9) Optional: If required, modify the annotations for the fluorescence channels. The default settings are shown below.

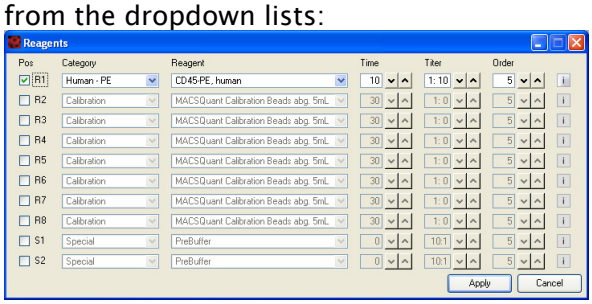


Autolabel tab

10) Optional: If autolabeling is required, add the relevant reagents by clicking on an <add…> checkbox.



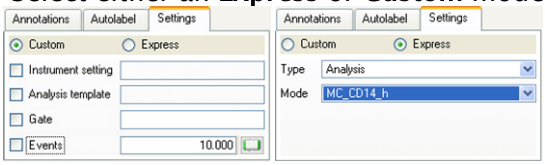
- Click an available checkbox 
- Select the reagent, reagent rack position, incubation time, titer and order from the dropdown lists:



See section 6.6 for information about reagent management.

Settings tab

11) Select either an **Express** or **Custom** mode of analysis:



For Custom mode analysis click the checkbox associated with the Instrument settings, Analysis template, Live gate and Events options to activate these features.

- Instrument setting: previously saved instrument calibration and compensation settings can be loaded and applied by checking the adjacent box.
- Analysis template: previously saved cell analysis templates can be loaded and applied by checking the adjacent box.
- Live gate: check the adjacent box to activate live gating – a live gating strategy can be saved as an analysis template for future use.
- Events: check the adjacent box to stop data acquisition after a defined number of events is obtained, in this example 10,000 events.

Note: It is recommended to limit measurement by volume. Volumetric measurements allow for absolute cell counting.

12) Ensure that reagents, samples and buffers are correctly positioned. Check that the waste bottle is empty.

Note: It is assumed that the instrument hardware and settings have been correctly calibrated. The analyzer should have been correctly compensated.

13) Click **Start Measurement**,  , to start acquisition.

Table 6.4 shows an overview of the above described procedure for defining an experiment.

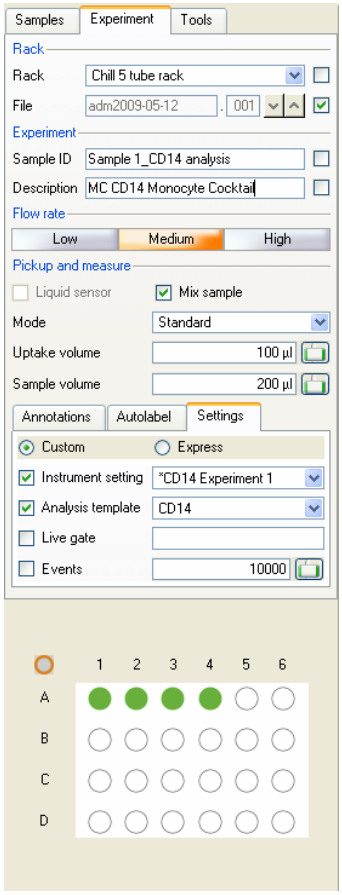
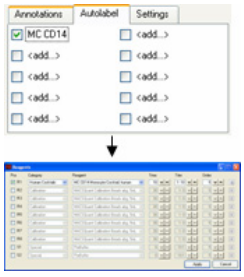
	Selected setting	Description
	Chill 5 tube rack	A chill 5 tube rack will be used for this analysis.
	File name “adm2009-05-12.001”	Filename is automatically generated. To enter a name manually, uncheck the associated box to the right.
	Sample ID and Description	The Sample ID and Description are text fields entered by the user.
	Flow rate	Medium flow rate was selected (50 □L / min).
	Mix sample checked (activated)	The samples will be premixed by the uptake needle.
	Custom mode	Custom mode is enabled. A previously saved Instrument setting and Analysis template were selected for the analysis. If the instrument settings option is not checked, the last successful calibration will be used.
	Events	The events checkbox was not selected. Absolute cell counting is therefore available since you will not acquire data after 10,000 events.
	Rack configuration	Samples in row A (A1 to A4) will be measured.
	Autolabel tab.	MACS Control(MC) CD14 Monocyte Cocktail was selected and the vial placed on rack position one.

Table 6.4 Example of an experiment definition for analysis of four samples with the MACS Control CD14 Monocyte Cocktail.

6.4 Switching to Express mode from Custom mode

1) In Custom mode click **Express mode** button in the top right-hand of the navigation bar.

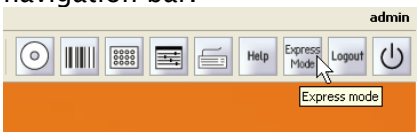


Figure 6.12 Switching to Express mode from Custom mode.

2) The MACSQuantify Software window will change to the Express mode.

Note: If windows are active in the Custom mode (e.g. analysis window), the user will be prompted to confirm this action. Click **Yes** to continue and **No** to cancel the switch.

Note: Any active work will **NOT** be transferred to the Express mode. All data or settings must be saved before switching to Express mode.

6.5 Printing in Custom mode

The MACSQuantify Software uses installed windows printer drivers to print active workspaces.

Note: The HP Universal Print driver has been installed on the MACSQuant Analyzer and has been tested with the following printers:

Hp Laserjet - P2055d; P3005n; CP1515n; PC2025n
Hp Officejet Pro 8000

For a complete list of printers compatible with the HP Universal Print driver, please visit: www.hp.com/go/upd. Please note the only the above mentioned printers have been tested with the MACSQuant Analyzer.

Note: It is also possible to print to a network printer. Please contact your MACSQuant Analyzer administrator or Miltenyi Biotec technical support for more information.

To print active workspaces:

1) Open the desired workspace or analysis window.

2) Click  .

3) Select the desired printer. Click **Print**.
The printer can be networked to or directly connected to the MACSQuant Analyzer or to the PC running the MACSQuantify Software.

The active workspace is printed as shown below.

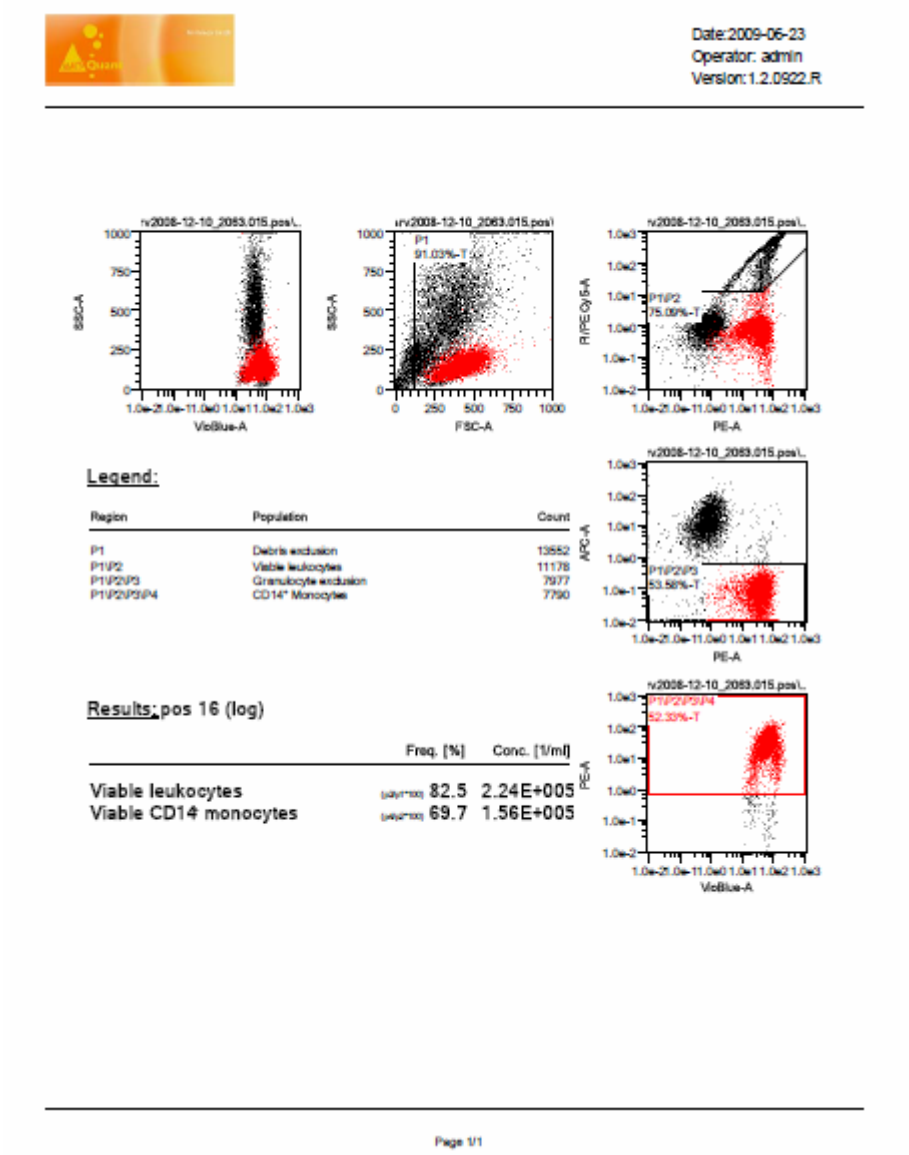


Figure 6.13 Half-size example print-out of data analyzed by the MACSQuantify Software in Custom mode.

6.6 Reagent management

Reagents are positioned on the reagent rack. There are four positions for reagent vials on the MACS Reagent Rack 4. Reagents can be entered using the 2D code reader or manually using the **Reagents** window.

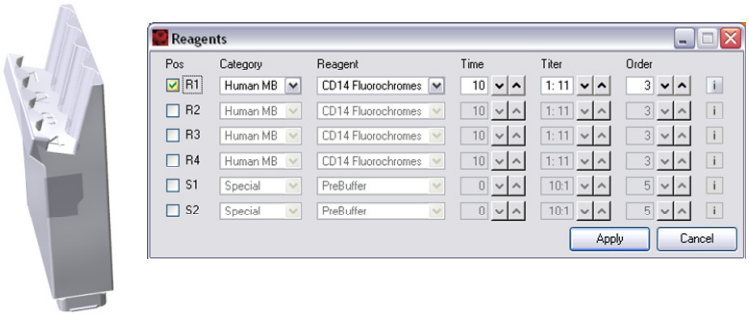


Figure 6.14 The reagent rack. Reagent positions 1, 2, 3 and 4 are clearly marked on the MACS Reagent Rack and correspond to positions R1, R2, R3 and R4 of the MACSQuant Analyzer reagent management window.

6.6.1 An overview of the MACSQuant Analyzer reagent management window

The MACSQuant Analyzer reagent management window allows users to select reagents from a dropdown list and assign reagents to a position on the reagent rack. The available options are described below:

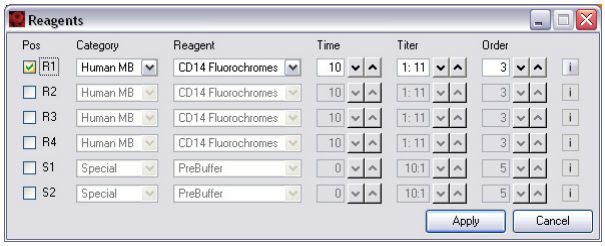


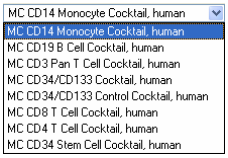
Figure 6.15 The MACSQuant Analyzer reagent management window.

- **Pos**: Use this checkbox to assign reagents to rack positions R1, R2, R3, R4, S1 or S2.
    - R1–R4 positions are located on the MACS Reagent Rack 4.
      S1 and S2 positions are denote as "Special" positions, where the Running Buffer is taken directly from the buffer bottle.

      Note: The MACS MiniSampler must be correctly installed to view these options.

- **Category**: Reagents are categorized according to species, conjugated fluorochrome and purpose. The current categories follow:
    - Calibration: MACSQuant Calibration beads for calibration of the instrument settings
    - Species and conjugated fluorochrome, e.g., Human – APC, Mouse – PE
    - Isotype control: isotype control antibodies are raised against non-mammalian epitopes and can be therefore used as a negative control for non-specific binding.
    - MACS Comp Reag. (MACS Compensation Reagents): These reagents are used to correct the inherent spectral overlap between excitation and emission wavelengths of fluorochromes.
    - Universal (for generic labeling strategies using "Tags" such as biotin of His)

- **Reagents**: A dropdown list of available reagents is displayed in accordance with the selected category, for example, the following reagents are available for the category "Human Cocktails".



- **Time**: For autolabeling an incubation time is given. The recommended incubation time is automatically shown in a black font type. Experienced users may wish to change the incubation time using the adjacent arrows (); note that non-recommended times will appear in a red font type, e.g., 11.
- **Titer**: For autolabeling a recommended label to sample titer is given. The recommended titer is automatically shown in a black font type. Experienced users may wish to change the titers using the adjacent arrows (); note that non-recommended titers will appear in a red font type, e.g., 1: 11.
- **Order**: Signifies the order at which this reagent will be used during cell processing.
- : Further information about the reagent is shown when this icon is activated.

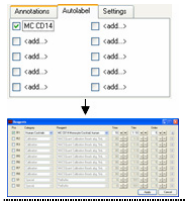
6.6.2 Selecting and assigning reagents manually using the MACSQuantify Reagents dialog box

Note: It is recommended to use the barcode reader to scan reagents (see section 6.6.3). This protects the user against making incorrect reagent entries. However, if the reagent label or datasheet insert is damaged, it may be necessary to manually select reagents using the **Reagents** dialog box.

To select regents manually:

1) Place a reagent onto the reagent rack noting its position.
2) Click **Edit** and **Reagents...**

Note: It is also possible to activate the reagent management window by checking an **Autolabel** <**add...**> box located in the **Experiment** window.



3) Check the desired reagent position R1, R2, R3, R4, S1 or S2. This must correspond to the correct position on the reagent rack.
4) Highlight a category that corresponds to the desired reagent using the **Category** dropdown list.

5) Highlight the desired reagent from the **Reagent** dropdown list.

6) Modify the incubation time (**Time**), label:sample titer (**Titer**) and order if required.

7) Click **Apply** to apply changes and close the window.

6.6.3 Scanning reagents with the 2D code reader

To enter regents manually into the reagents database:
The 2D code reader ("barcode reader") is used to scan reagent vials. Reagent vials are automatically recognized and logged by the MACSQuantify Software.

To scan reagents perform the following:

1) Click the activate code reader icon,  . The code reader will being blinking.

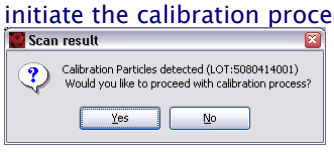
2) Present the reagent vial in front of the 2D code reader. Ensure the 2D code is facing the blinking code-reader light. The optimal reading distance is 0.5–2.5 cm from the code reader cover, tilt the vial as depicted in Figure 6.16.

Figure 6.16 Scanning a reagent using the MACSQuant Analyzer 2D code reader.

3) Scanned reagents are reported a MACSQuantify Software dialog box.

4) Place the reagent onto the corresponding position on the reagent rack.

Note: When scanning MACSQuant Calibration Beads the instrument will prompt to initiate the calibration procedure:



Note: When scanning MACS Reagents the MACSQuantify Software will prompt the user to place the vial(s) on the MACS Reagent Rack.

Note: If the code reader fails to recognize the 2D code enter the information directly into the MACSQuantify Software reagent management window; see section 6.6 for more details.

6.7 Multisample processing

Multisample processing is accomplished by use of the MACS MiniSampler in combination with the MACS Reagent Rack 4 and MACS Cooling Tube Racks (sample tube racks). Five different kinds of sample tube racks are available: Single tube rack, Chill 5, Chill 15, Chill 50 and Chill 96 allowing for processing of up to 96 samples in a single batch (see Table 6.5 for details). The MiniSampler can be configured to perform measurements from any sample position. Depending on the user's instructions, samples can be labeled with fluorochromes and/or measured.

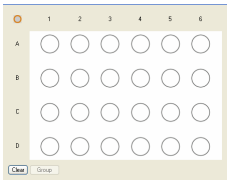
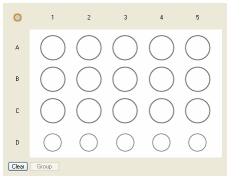
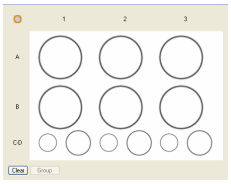
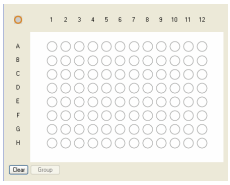
Rack type	Slots	Maximum number of samples	Option on MACSQuantify Rack drop-down list	Corresponding MACSQuantify Rack Graphic
Single tube rack	1 × 5 mL	1 (5 mL tube)	Single tube rack	Not applicable
Chill 5	24 × 5 mL	6 (5 mL tubes)	Chill 5 tube rack	
Chill 15	15 × 15 mL 5 × 5 mL	5 (15 mL tubes)	Chill 15 tube rack	
Chill 50	6 × 50 mL 3 × 15 mL 3 × 5 mL	3 (50 mL tubes)	Chill 50 tube rack	
Chill 96 rack/ 96 rack	96-well microtiter plate	96	96-well	

Table 6.5 Overview of the various rack types that may be used with the MACSQuant Analyzer. An appropriate rack should be used, depending on the sample number and volume.

6.7.1 Selecting a sample rack

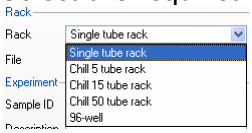
Note: This information is also relevant to section 6.3.6 (Define an experiment in Custom mode) of this manual.

To select a sample rack:

1) Click on the **Experiment** tab, 

.

2) Select the required rack type from the **Rack** dropdown list.



3) The corresponding rack template will popup in an independent window and will also appear in the lower section of the **Experiment** tab window.

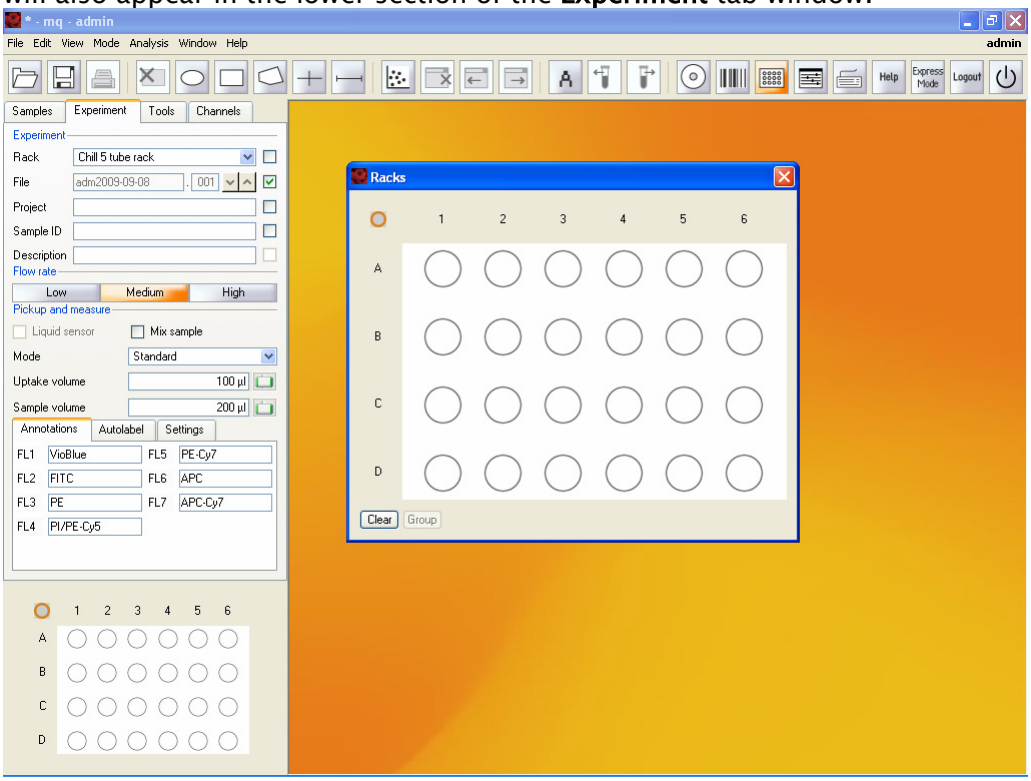


Figure 6.17 The Chill 5 tube rack template was displayed.

> **Note:** The popup rack window can be closed or opened at any time using by clicking the **Rack** icon: 

6.7.2 Configuring the sample rack for an experiment

> **Note:** This information is also relevant to section 6.3.6 (Define an experiment in Custom mode) of this manual.

Sample racks are represented graphically by the MACSQuantify Software. All rack positions are given by coordinates: columns are assigned numbers; rows are assigned letters.

To select a single rack position, use the left mouse button (single-click) to activate the desired rack coordinate; alternatively, the MACSQuant Analyzer touchscreen may be used.

Figure 6.18 Configuring a sample rack using the MACSQuant Analyzer touchscreen

- When a rack position is selected for the first time by a single mouse click, the following status is reported: .
- By re-selecting the same rack position the status changes from to .

An explanation of the various rack configurations for single sample positions is given by Table 6.6.

User action with left mouse button or fingertip action on touch-screen	Effect	Details
None		Default open circle indicates no operation: Clear
Single click on circle/single finger-touch		Closed green circle with orange rim: Sample selected for measurement. Orange circle indicates that the sample is selected and any alterations made to the measurement strategy (e.g. labeling) will only apply to sample positions with this designation.
Double click on circle/double finger-touch		Closed green circle: Sample selected for measurement
None		Closed blue circle: Measurement in progress
None		Closed gray circle: Measurement finished
None		Closed yellow circle: Processing of sample has commenced, e.g., sample has been labeled and incubation is underway

Table 6.6 Summary of rack configurations

More than one rack position can be selected at once. An entire rack, row or column can be selected or deselected. Furthermore, samples can be grouped together using the **Group** function. After sample acquisition, grouped samples can be analyzed together

in a single dataset/analysis window. Refer to Table 6.7 for information on the selection of multiple rack positions.

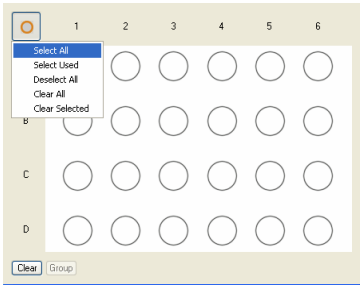
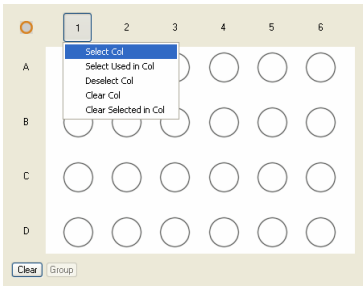
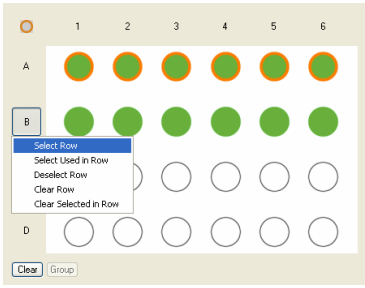
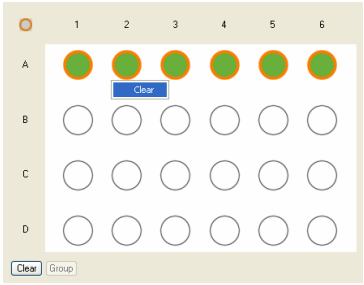
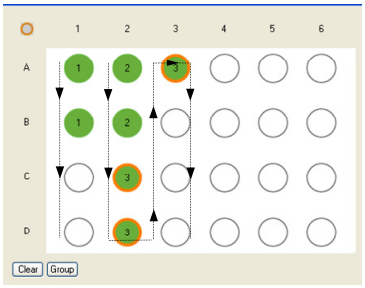
User action to select multiple sample positions	Effect	Details
Single right click of the multiple sample menu button.		Use this button to change the settings for all rack positions. Note: In order to set all rack positions to allow Measurement and modification of the experiment strategy (e.g. labeling): Click Select All, followed by, Deselect All
Single right click on column header		Selection/deselection of an entire sample column.
Single right click on row		Selection/deselection of an entire sample row. In this example: Row A is selected for sample labeling and measuring. Row B is selected for sample measurement only.
Single right click over a single rack position		Right click over a single rack position to completely clear this position. In this example, position A2 will be cleared.
Grouping function		Only rack positions that are adjacent and in columns can be grouped. To group several adjacent rack positions: 1) Select the rack positions, 2) Click **Group**, See 6.8.3 grouping sample for more information.

Table 6.7 An overview of the possible configurations for rack positions.

To configure a sample rack:

1) Click on a sample position(s) using the left mouse button or touchscreen. An entire row, column or table can be selected using, see Table 6.7 for more details.

2) Use left mouse button or touchscreen to toggle between and .

a. : designed sample will be measured and associated "**Experiment**" definitions for this sample can also be modified using the **Experiment** tab (e.g. sample name, labeling strategy, uptake volume etc.).

b. : designed sample will be measured and associated "**Experiment**" definitions can not be changed.

3) Use the **Experiment** tab to change the **Experiment**, **Flow rate** and **Pickup and measure** options as required.

> **Note:** To reiterate: Only sample positions with status " " can be "programmed" using the Experiment tab.
>
> **Note:** For more information about defining an experiment refer to section 6.3.6 (Define an experiment in Custom mode).

4) To close the popup rack window click the Rack icon: .

5) Before starting the run check that:

a. Experiment definitions are correctly assigned to each rack coordinate and that each sample is correctly positioned on the Chill Rack.

b. Sufficient quantities of reagents and buffers are provided. Ensure that the waste bottle is empty.

c. The reagents have imported and assigned to a position on the MACS Reagent Rack (see section 6.6).

d. The instrument is correctly calibrated and compensated (see sections 3.6 and 3.7).

6.8 Defining an experiment with multisample processing: A work-through example

6.8.1 Background

In the following example CD14$^+$ cells from three human PBMC samples were enriched using the autoMACS Pro Separator in combination with the Monocyte Isolation Kit II. It was important to evaluate the outcome of these cell separations using flow cytometry.

This can be easily and quickly achieved using the MACSQuant Analyzer in combination with the MACS Control (MC) CD14 Monocyte Cocktail (human).

Three samples were placed on rack positions A1, A2 and A3 of a Chill 5 tube rack.

- Cells in sample tube positions A1 and A2 were analyzed using the MACS Control (MC) CD14 Monocyte Cocktail in the **Express** mode.
- Cells in sample tube position A3 were analyzed in the **Custom** mode using an analysis template. The default instrument setting was used, i.e. the most recent instrument setting which is defined as **Default(*)**.

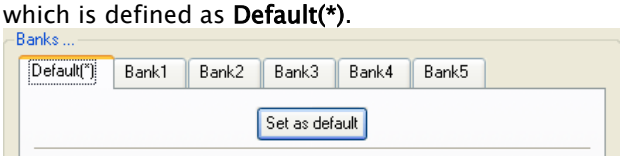


Figure 6.19 The calibration bank settings are accessible

> **Note:** See sections 3.6 and 6.12 for more information on Instrument settings and analysis templates, respectively.

6.8.2 Rack configuration and sample definition

1) Choose **Chill 5 tube rack** from the **Experiment** tab.

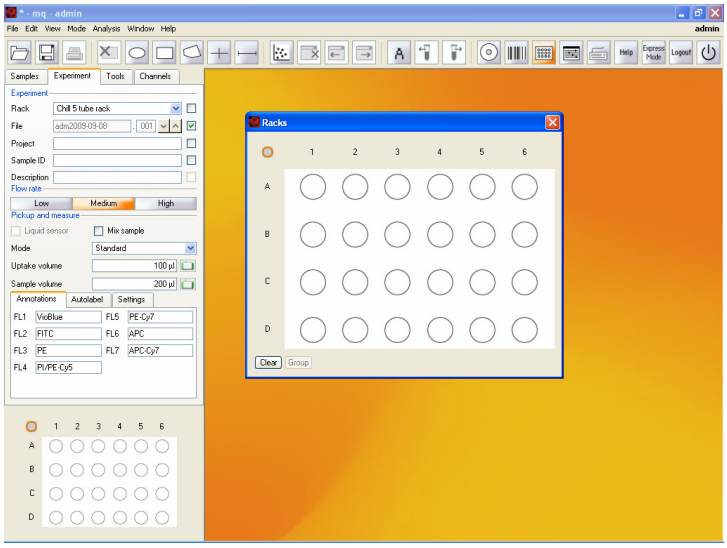


Figure 6.20 Chill 5 tube rack was selected for multisample labeling

2) Left-click once on rack coordinates A1 and A2.

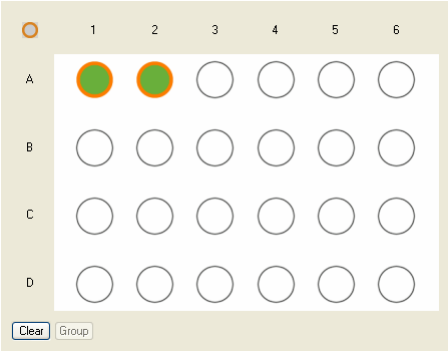


Figure 6.21 "Measure and select": The settings for sample positions A1 and A2 may be modified , e.g., a labeling strategy may be applied

3) Define the experiment settings for positions A1 and A2 as shown below (see section 6.3.6 for more details on defining experiments).

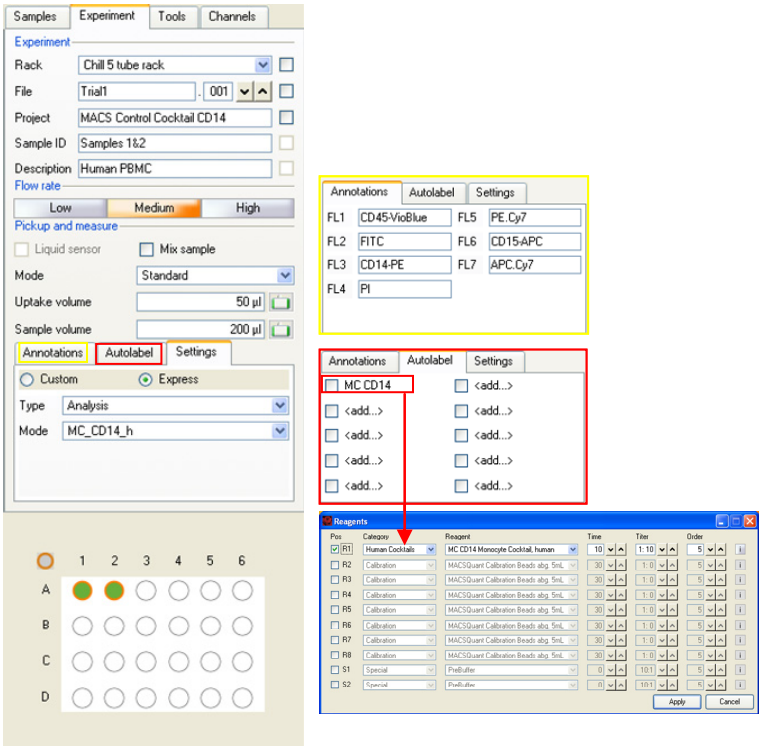


Figure 6.22 Experiment definition for sample positions A1 and A2. Note that reagent vial MC CD14 Monocyte Cocktail (human) was defined to position one of the MACS Reagent Rack (Red box). Default annotations (yellow) were used for the analysis channels.

4) Rack positions A1 and A2 are defined.

5) Left-click once on the rack coordinate A3 to define experiment settings for this position.

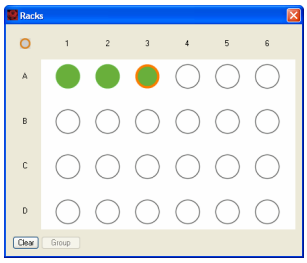


Figure 6.23 Rack position A3 was chosen experiment definition.

Note: Rack positions A1 and A2 are now saved i.e., ●.

6) Define the experiment settings for position A3 as shown below (see section 6.3.6 for more details on defining experiments).

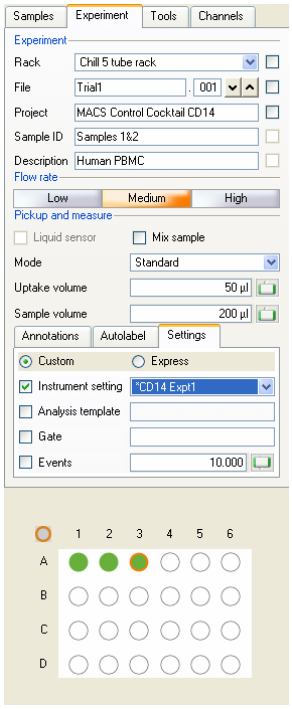


Figure 6.24 The sample in rack position A3 will be analyzed in custom mode using a previously saved Instrument setting and Analysis template.

7) The samples have been assigned to a rack positions and defined for analysis.

6.8.3 Rack configuration and sample grouping

Sample grouping can be made before acquisition or afterwards during data analysis. Refer to section 6.14 for information about sample grouping after data analysis.

What is the benefit of sample grouping?

The maximum sample volume that can be acquired in a single step by the MACSQuant Analyzer is 450 µL. There are occasions when the sample size is of course greater; aliquots of the sample must therefore be spanned over two or more tubes.

By grouping these samples, the acquired data will be consolidated into a single file on the hard drive, which can also be analyzed in a single data file or analysis plot.

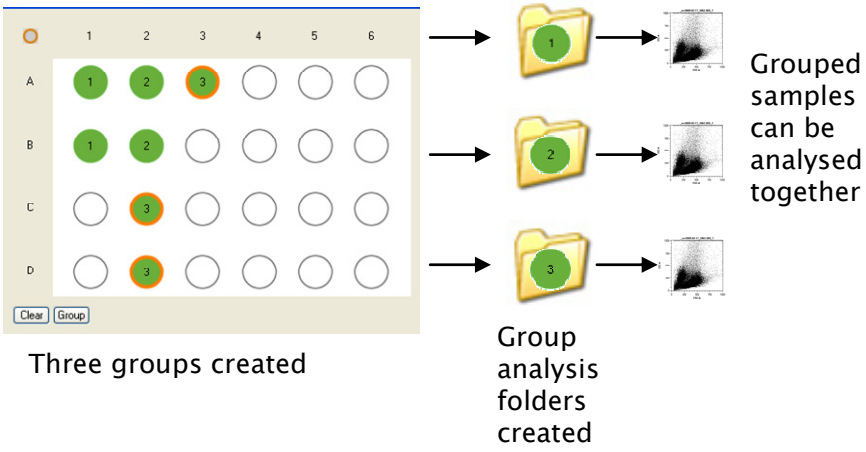
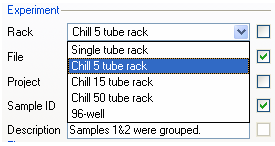


Figure 6.25 Schematic of the grouping process

To group samples:

1) Using the **Experiment** tab, select the desired rack from the drop-down list.



2) Click  to open the corresponding rack dialog box. Alternatively use the keyboard shortcut: **Ctrl+Alt+R**.

3) Select sample positions for grouping. These sample positions must be in a column, for example:

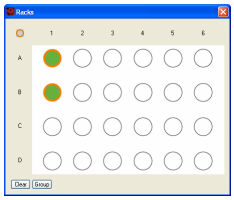


Figure 6.26 Only samples in a column can be selected. In this example column 1 was selected and adjacent sample positions A1+B1.

4) Click **Group**,  .

5) Enter the sample information using the **Experiment** tab.

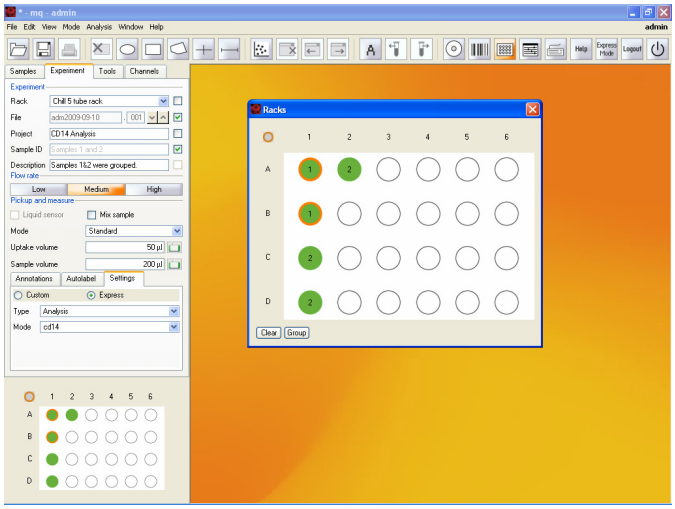
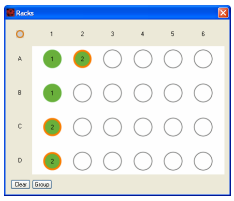


Figure 6.27 The above sample information applies to group 1 (rack coordinates A1 + B1) only.

6) Click on additional desired rack positions to perform further grouping. Add sample information as required.



7)  – Close the Rack dialog box.

6.9 Working with data files in Custom mode

Refer to the sections “Opening files”, “Saving files”, “Importing files” and “Exporting files” for immediate instructions on handling these file types. If you are unfamiliar with the user interface or options associated with handling files, read the following information “Introduction to file handling”.

6.9.1 Introduction to file handling

This section describes how data files can be opened, saved, and backed-up in **Custom** mode. Data files may be stored to and therefore opened from a **Public**, **Private** or **External** file location.

- Public files are located on the local hard drive of the MACSQuant Analyzer (or personal computer) and are accessible by all users.
- Private files are located on the local hard drive of the MACSQuant Analyzer (or personal computer) and are only accessible by the logged-in user account.
- External files are located on an independent file storage device which is connected to the MACSQuant Analyzer (or personal computer) via the USB port i.e. a memory stick.

The default window for saving and opening data files is composed of the following tabs:

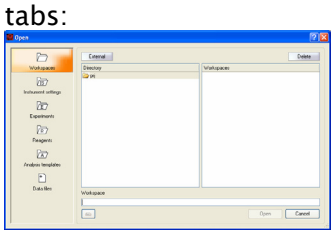
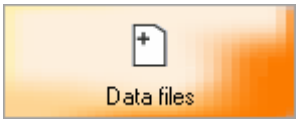


Figure 6.28 The default window for opening and saving various file types.
Note: The availability of these tab options is dependant on the user profile (Custom user, Express user or administrator) and whether data/settings are being saved or opened.

Tab option	Description
	The Workspaces tab allow users to save user an entire workspace which is composed of instrument settings, experiment and reagent definitions, and an analysis template with accompanying data.
	Instrument settings are compensation and calibration parameters for the MACSQuant Analyser. These parameters are important for data analysis and are vital to maintain standardized results over time and from instrument to instrument. The MACSQuantify Software can open and save instrument settings. These settings can be applied to acquired data and thus this useful feature allows users to perform recompensation after data acquisition. The **Instrument settings** may be saved but not opened in **Express** mode.
	Experiment definitions can be saved for future use. Reagent type and corresponding Reagent Rack 4 positions, sample rack type and corresponding Chill Rack sample positions, the analysis mode and sample processing definitions (e.g. labeling strategy) comprise experiment definitions.
	Reagent type and position on the reagent rack can be saved using the **Reagents** tab. The **Reagents** tab is not available in Express more.
	**Analysis templates** are predefined analysis layouts for data acquired by the MACSQuant Analyzer. The templates are creating by defining a gating strategy with associated plots, histograms, tables and statistics. Administrators and Custom users can customize and save templates for reuse. Express users cannot create or modify **Analysis templates**.
	**Data files** can be saved to a **Public**, **Private** or **External** file location by all users. MACSQuant Data Data (MQD) is the standard file handling format, however, the MACSQuantify Software can also import Flow Cytometry Standard (FCS) file types.

6.9.2 Opening files

1) Click  to open the **Open** window.

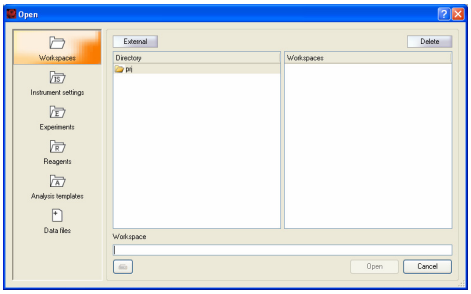


Figure 6.29 Custom mode users and administrators are able to open Workspaces, Instrument settings, Experiments, Reagents, Analysis templates and Data files.

2) Click on the desired tab, for example, the **Experiment** tab or **Data file** tab to open an experiment definition or data files, respectively.

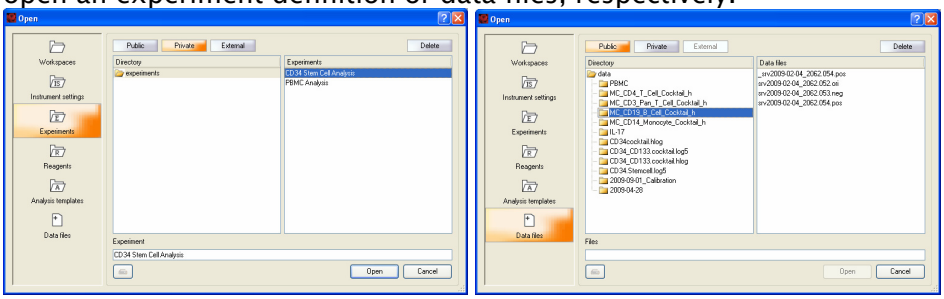


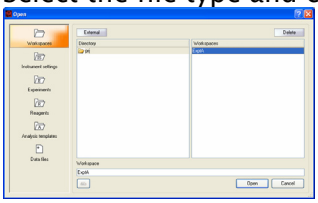
Figure 6.30 Highlight the desired tab to view available files. The "Experiment" (left) or "Data files" (right) tabs were selected in this example. Note: Multiple data file types can be selected and opened at once (right).

To open workspaces:

1) Highlight the **Workspace** tab on the **Open** window.

2) Highlight the file location **External** to open files from external media, e.g., a USB memory stick. Files are opened from the default **Private** location.

3) Select the file type and click **Open**,  .

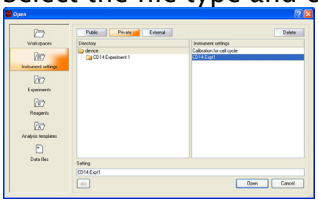


To open Instrument settings:

1) Highlight the **Instrument** tab on the **Open** window.

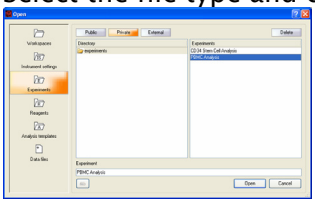
2) Highlight the file location: **Private**, **Public** or **External**.

3) Select the file type and click **Open**, Open.



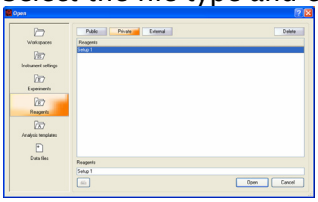
To open experiment definitions:

1) Highlight the **Experiment** tab on the **Open** window.
2) Highlight the file location: **Private**, **Public** or **External**.
3) Select the file type and click **Open**, Open.



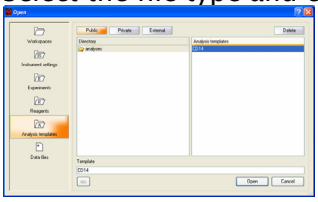
To open reagents:

1) Highlight the **Reagents** tab on the **Open** window.
2) Highlight the file location: **Private**, **Public** or **External**.
3) Select the file type and click **Open**, Open.



To open analysis templates:

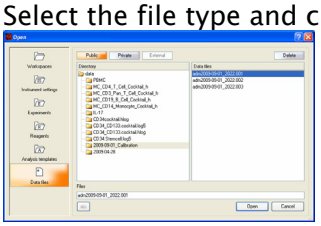
1) Highlight the **Data files** tab on the **Open** window.
2) Highlight the file location: **Private**, **Public** or **External**.
3) Select the file type and click **Open**, Open.



To open data files:

1) Highlight the **Data files** tab on the **Open** window.
2) Highlight the file location: **Private**, **Public** or **External**.

3) Select the file type and click **Open**, Open.



Note: Several files can be selected and opened at once.

6.9.3 Saving files

1) Click to open the **Save** window.

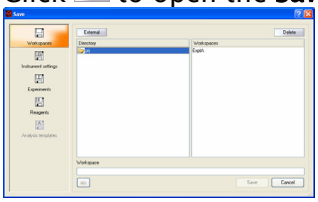


Figure 6.31 The Save window. Custom users and administrators are able to save Workspaces, Instrument settings, Experiments, Reagents and Analysis templates. Analysis templates can only be saved when the Analysis mode icon ( ) is activated.

2) Click on the **Workspace** tab, **Instrument settings** tab, **Experiments** tab, **Reagents** tab or **Analysis template** tab to save the relevant file type.

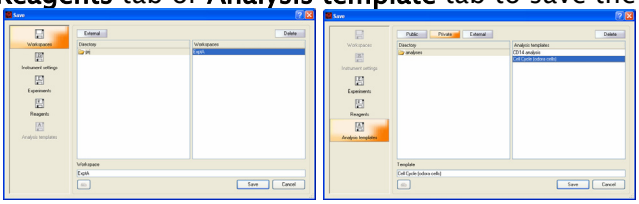


Figure 6.32 Saving file types. Left: "Workspaces", "Instrument settings", "Experiments" or "Reagents" file types can be saved. Right: Analysis mode was activated ( ) in order to save "Analysis templates".

3) Using the keyboard enter the file name.

4) Click **Save** to save files.

6.9.4 Importing files

MACSQuantify Software can import files in Flow Cytometry Standard (FCS) Formats. MACSQuantify Software data and instrument settings can also be imported from an external file location, for example, a USB memory stick.

To import FCS files perform the following steps:

1) Insert a memory stick or external media into the MACSQuant Analyzer USB port. Alternatively, ensure that the MACSQuantify Software has intranet access to the file location.

2) Click **File** and **Import FCS file**.

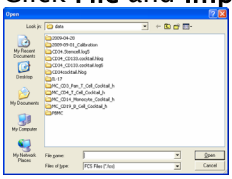


Figure 6.33 Importing an FCS File.

3) Navigate to the file using windows tabs. Highlight the file and click **Open**.

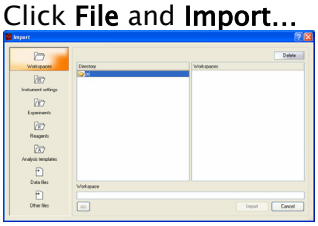
4) The file will be imported.

To import MACSQuantify Software files from an external source perform the following steps:

1) Insert the external media to the MACSQuant Analyzer USB port or computer USB port.

Note: If no external device is attached to the MACSQuant Analyzer or personal computer the following error will be displayed:

2) Click **File** and **Import...**



3) Select the file type: **Workspaces**, **Instrument settings**, **Experiments**, **Reagents**, **Analysis templates**, **Data files**.

4) Click **Open**.

5) The file(s) will be imported.

6.9.5 Exporting files

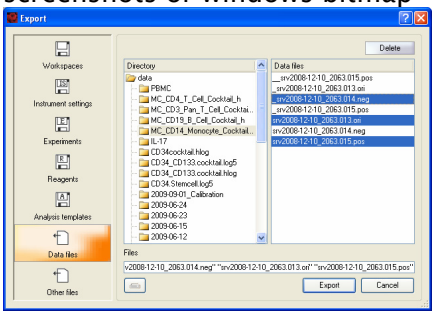
MACSQuantify Software data and instrument settings can be exported to a USB memory stick.

To export MACSQuantify Software files to an external source perform the following steps:

1) Insert the memory stick to the MACSQuant Analyzer USB port or computer USB port.

Note: If no external device is attached to the MACSQuant Analyzer or personal computer the following error will be displayed:

2) Navigate to and highlight the file for export. **Workspaces**, **Instrument settings**, **Experiments**, **Reagents**, **Analysis templates**, **Data files** and **Other files** (e.g. screenshots or windows bitmap <bmp> files) can be exported.



3) Click **Export**.

4) The file will be exported.

6.10 Data backup and restore in Custom mode

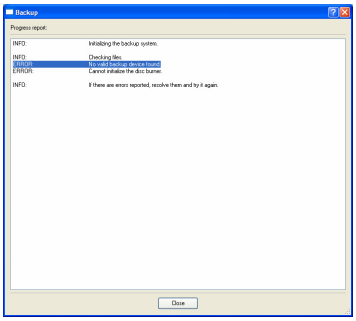
It is recommended that data is regularly backed-up to an external location. Data can backed-up to a network drive, USB memory stick or DVD. Administrators can configure data backup settings. Please contact your administrator for more information or refer to sections 6.10.4 and 6.10.5 of this help guide.

Note: Before performing **Backup**, ensure that the desired backup media is accessible to the MACSQuantify Software.

Backup media

The backup procedure ( ) searches for backup media in the following order:

1) A designated folder located on a local area network: this must be setup by an administrator with assistance from Miltenyi Biotec technical support.
2) A memory stick attached to the USB port on the MACSQuant Analyzer.
3) A rewritable DVD.
4) If none of the above are found, the MACSQuantify Software reports an error: No valid backup devices found.



6.10.1 To perform a backup to a rewritable DVD

1) Ensure no USB stick is installed and that no network drive has been defined as the default location for backup files. Please contact your administrator for further advice.

2) Insert a rewritable DVD into the MACSQuant Analyzer DVD drive. Only DVD-R or DVD-RW media may be used. DVD+RW and CD media types are not currently supported.

3) Wait for 10-20 seconds after inserting the DVD into the drive.

4) Click the backup icon located on the top menu bar,  .

5) The files will be written to DVD.

> **Note:** Depending on the amount of data, the backup procedure may take several minutes. When the progress bar displays 100% the MACSQuantify Software will verify the data once again; this may take a few minutes to complete.
>
> **Note:** At this stage data will NOT be deleted from the MACSQuant Analyzer, the data is only copied to DVD.

6) Insert the backup DVD into the destination DVD-drive of an independent personal computer on which the MACSQuantify Software is preinstalled. This computer can be used for data analysis.

7) Start and login to MACSQuantify Software on the personal computer.

8) Click restore  .

> **Note:** MACSQuant Analyzer data will be **copied** to the local drive of the personal computer. After a successful data transfer, the copied data will be "marked" as successfully copied on the DVD.
>
> **Note:** When performing a future data backup on the MACSQuant Analyzer, ensure that this backup DVD is used.

Performing subsequent MACSQuant Analyzer backup

9) Insert the **designated** MACSQuant Analyzer backup DVD.

10) Click backup,  .

> **Note:** The MACSQuant Analyser software (MACSQuantify) will identify data 'marked' on the DVD as successfully transferred to another computer. This corresponding data will be deleted off the MACSQuant Analyzer hard-drive and DVD before continuing with the backup procedure.

11) After backup is finished, remove the DVD from the drive.

12) Transfer the data to an independent personal computer as described above.

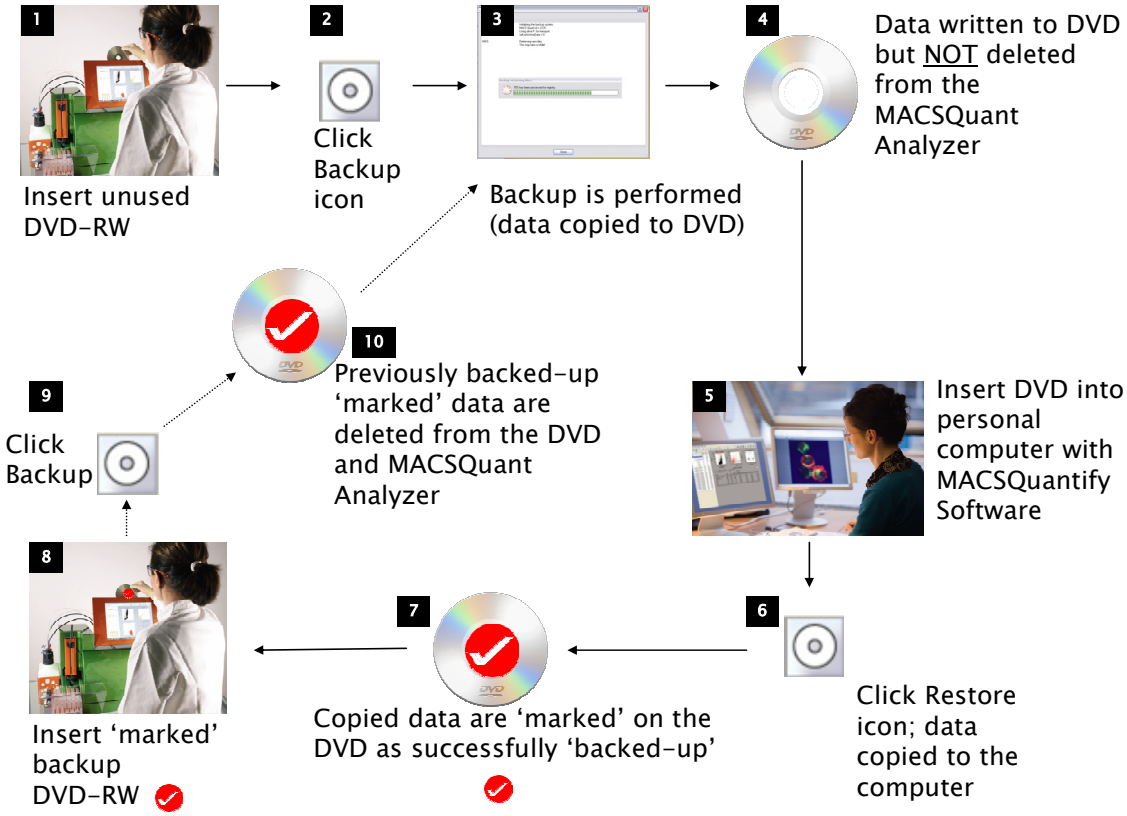


Figure 6.34 Schematic of the MACSQuantify Software/ MACSQuant Analyzer DVD backup procedure.

6.10.2 To perform backup to a USB memory stick

1) Ensure that no network drive has been defined as the default location for backup files. Please contact your administrator for further advice.

2) Insert a memory stick into the MACSQuant Analyzer USB port or USB port a personal computer. Wait a few seconds.

3) Click  .

4) The files will be automatically written to the USB memory stick.

Restoring files from a USB memory stick to a personal computer

5) Start MACSQuantify Software and login to a user account.

6) Insert the memory stick into a USB port of a personal computer with MACSQuantify Software installed.

7) Click **File** and **Import**...

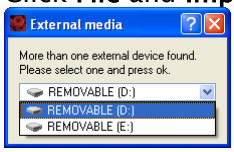
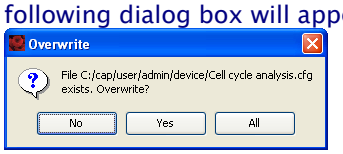


Figure 6.35 If more than one external USB is attached you will be prompted to selected the correct external device.

8) Use the dialog box to select the type of file for import e.g. Workspace, Instrument settings, Data files etc.

> **Note:** If a copy of the imported file already exists on the personal computer, the following dialog box will appear:
>
> 
>
>
> To overwrite a single file click **Yes**. To overwrite all files for import, click **All**. **No** aborts the procedure.

9) Highlight the file(s) and click **Import**.

> **Note:** The imported files are copied to MACSQuantify Software. It is necessary to delete files off the memory stick using windows explorer.
>
> **Note:** It is of course also possible to simply move files from the memory stick to a personal computer using windows explorer.

6.10.3 To perform backup to network drive

1) Please contact your administrator if a network drive has not been configured for backup.

> **Note:** If a network drive is not configured, the MACSQuant Analyzer software (MACSQuantify) will search for USB and DVD backup media instead.

2) Click  .

3) The files will be automatically written to the network drive.

6.10.4 Configuring data backup settings (administrators only)

1) Click **Edit**, **Options**, **Software** and **Backup**.

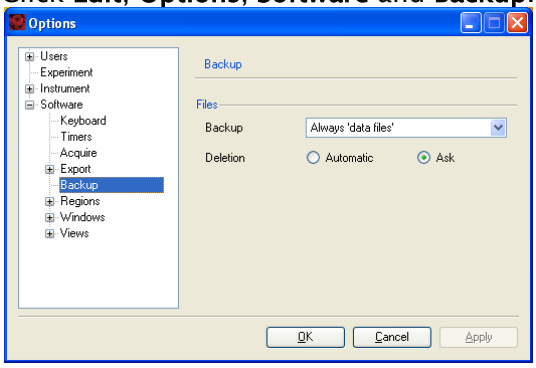


Figure 6.36 Changing the default file backup settings.

2) Use the dropdown list and/or radio buttons to activate/deactivate a feature:
Files Backup:

   a. **always ask** – before performing a backup the user is prompted to verify which file types are to be backed-up.

   b. **always 'all files'** – all files are always backed-up, there is no user prompt to verify this procedure.

   c. **always 'data files'** – only data files are backed-up, there is no user prompt to verify this procedure.

3) **Files Deletion**:

   a. **automatic** – automatically overwrite or delete files during backup.

   b. **ask** – the user is prompted to verify deletion or overwriting of files during backup.

4) Click Apply to implement changes. Click OK to close the window.

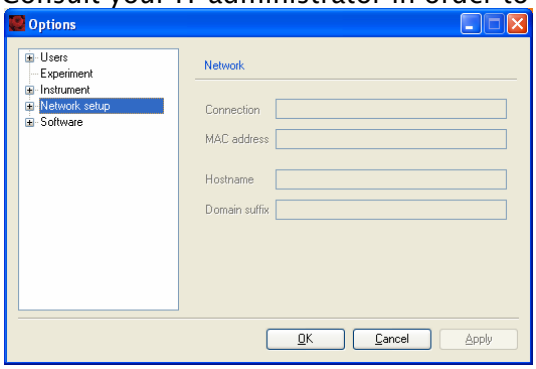
6.10.5 Configuring network settings (administrators only)

In order to have remote assistance and data backup to a network location it is necessary to configure the MACSQuant Analyzer network configuration.

To configure the MACSQuant Analyzer for network access:

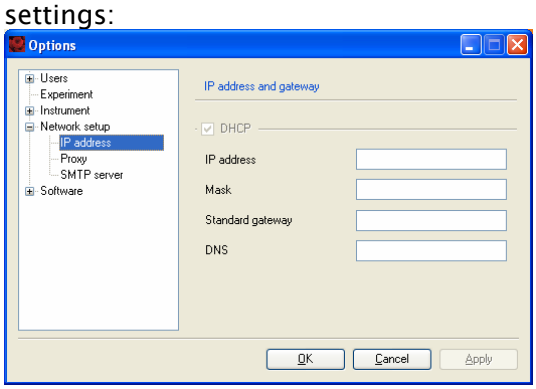
> **Note:** It will be necessary to contact your local network administrator. I may also be necessary to seek assistance from your Miltenyi Biotec technical support.

1) Click **Edit**, **Options**, **Network setup**.
2) Consult your IT administrator in order to configure the following settings:



3) Expand the tree Network setup 

and configure the following settings:



4) Click **Apply** to implement changes. Click **OK** close the window.

6.11 Configuring the default user, instrument and software options

The **Options** menu is used to customize user, software and instrument settings. Custom users and administrators can customize software and instrument settings, Only administrators have permission to modify user settings. An explanation of the Options menu follows.

6.11.1 Changing the default user options

> **Note:** User options can only be modified by administrators.

User groups (Express, Custom, Administrator) are managed using the Users menu. File access permissions and the default location of files can be stipulated using this menu.

To assign properties to a user group:

1) Click **Edit** and **Options….**

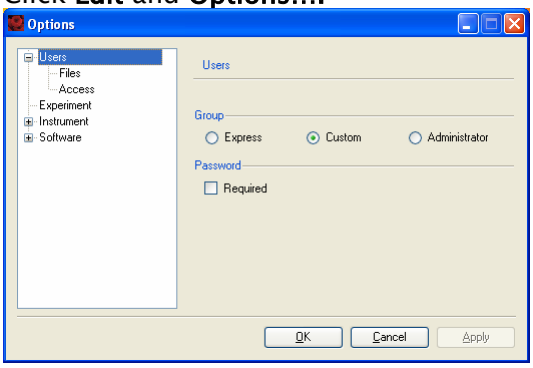


Figure 6.37 The Users options window.

2) Click the desired radio button: **Express**, **Custom** or **Administrator**.

3) Check the **Password** box if a passport is required for this group.

4) Click OK to save changes.

To assign properties to user file paths:

1) Click **Edit**, **Options…** and **Files.**

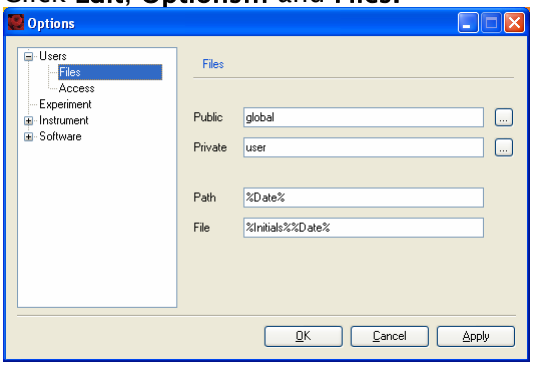


Figure 6.38 Changing default file paths for user groups.

2) **Public**: To change the location of the global folder click … and select an appropriate file location. To make a new folder click: Make New Folder

Private: To change the location of the private folder click … and select an appropriate file location. To make a new folder click: Make New Folder

3) Change the values between %% as required.

4) Click **Apply** to implement changes. Click **OK** close the window.

To assign a default naming conventions to user files and folders:

1) Click **Edit**, **Options**... and **Files.**

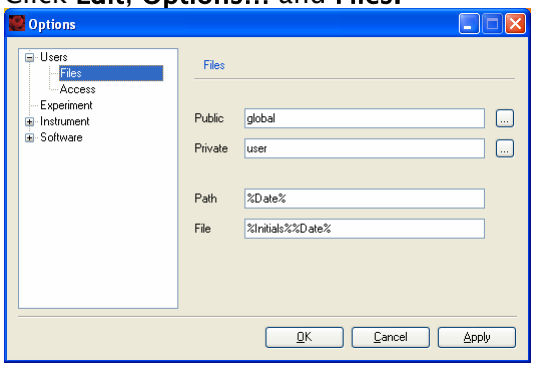
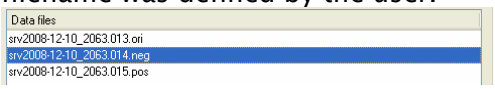


Figure 6.39 Changing the default naming convention for user folders and user filenames.

2) **Path**: The default naming convention for user folders is %Date%, i.e., new folders are creating according to the system date: YYYY-MM-DD.
File: The default naming convention for user files is %Initials%%Date%, i.e., new files are creating using a prefix of the users initials followed by the current system date. For example, filename "**srv2008-12-10**_2063.013.ori" was created by user "Srv" on the 10th December 2008. The remaining part of the filename was defined by the user.



3) Change the values between %% as required.

4) Click **Apply** to implement changes. Click **OK** close the window.

To assign user permission to user groups:

1) Click **Edit**, **Options**... and **Access.**

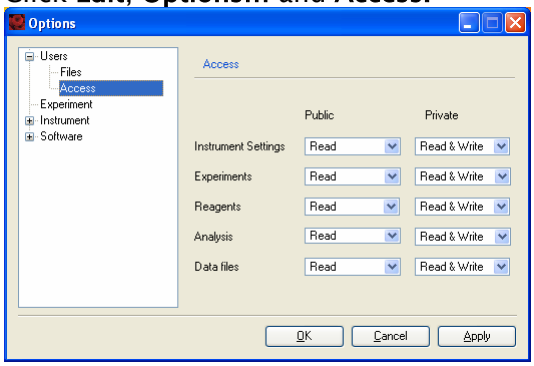


Figure 6.40 Changing default file paths for user groups.

2) Use the dropdown list for each access category to modify the read and write privileges as required.

3) Click **Apply** to implement changes. Click **OK** close the window.

6.11.2 Changing the default experiment options

Note: Experiment options can only be modified by administrators.

The default Experiment settings can be modified using the Experiment options menu.

To assign default Experiment settings:

1) Click **Edit**, **Options...** and **Experiment.**

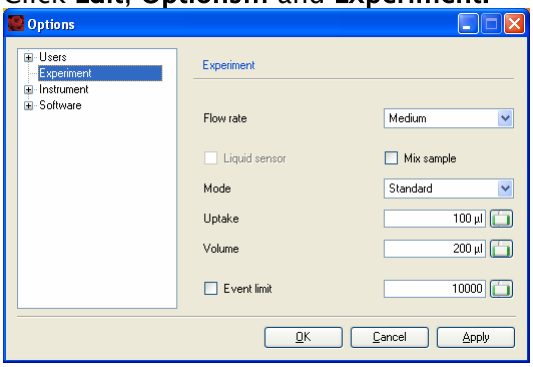


Figure 6.41 Changing default experiment settings.

2) Modify the default values for **Flow rate**, **Mode**, **Uptake**, **Volume**, and **Event limit** as required.

Note: It is not recommended to activate the Event limit setting: Event limit. Limiting data acquisition to the number of events prohibits volumetric cell counting.

3) Click **Apply** to implement changes. Click **OK** close the window.

6.11.3 Changing the default instrument options

The default Instrument options are the instrument name, instrument features and instrument annotations. Each are briefly discussed below.

To assign a default Instrument name:

Note: The default value is blank, i.e., no name is given.

1) Click **Edit**, **Options**... and **Instrument.**

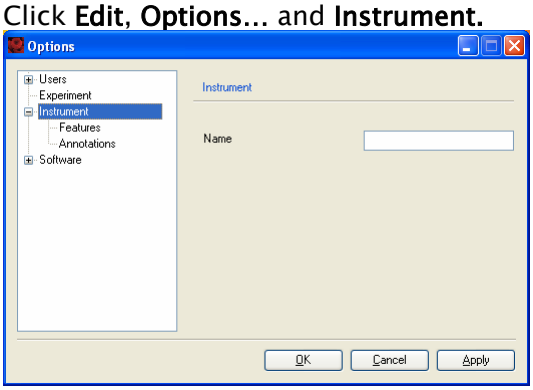


Figure 6.42 Changing the default instrument name.

2) Enter alphanumeric text into the **Name** field.

3) Click **Apply** to implement changes. Click **OK** close the window.

To assign a default Instrument features:

1) Click **Edit**, **Options, Instrument** and **Features.**

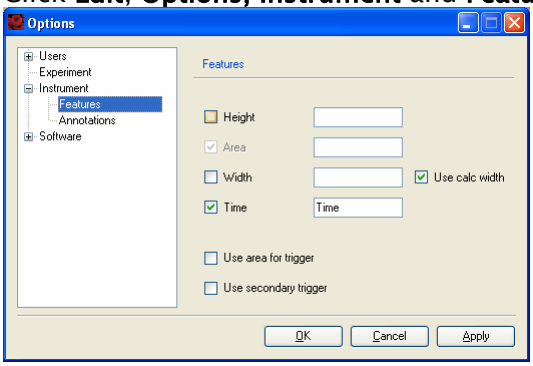


Figure 6.43 Changing the default instrument features.

2) Click the checkbox to activate the corresponding feature.

3) Enter desired alphanumeric text into the corresponding field.

4) Click **Apply** to implement changes. Click **OK** close the window.

To assign a default instrument Annotations:

Instrument annotations are used to provide additional description of the seven analysis channels that are available on the MACSQuant Analyzer. Data analysis and interpretation is arguably easier when suitable annotations are used, for example: FL2 channel could be annotated as “FITC” as this fluorochrome is detected in this channel.

1) Click **Edit**, **Options, Instrument** and **Annotations.**

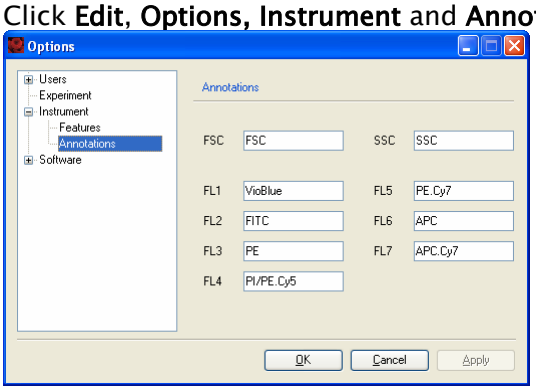


Figure 6.44 Changing the default instrument annotations.

2) Enter desired alphanumeric text into the corresponding textbox.

3) Click **Apply** to implement changes. Click **OK** close the window.

6.11.4 Changing the default software options

The following default software options can be modified: keyboard, timers, acquisition settings, export and backup settings and the default display settings for regions, plots, histograms and tables. Each software option is briefly discussed below.

To assign default settings for the Keyboard:

> **Note:** Feature only applicable to the MACSQuant Analyzer. This function is not available to MACSQuantify Software installations on personal computers.

1) Click **Edit**, **Options, Software** and **Keyboard.**

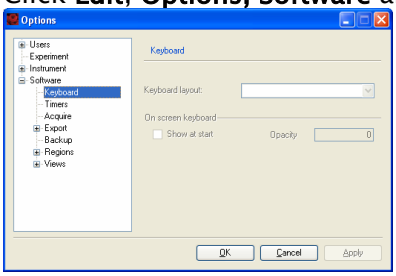


Figure 6.45 Changing the default keyboard settings.

2) Use the dropdown list to select a keyboard format. The opacity can be modified as required.

3) Click checkbox **Show at start** to automatically activate the touchscreen keyboard at MACSQuant Analyzer startup.

4) Click **Apply** to implement changes. Click **OK** close the window.

To assign default settings for Timers:

Note: Feature only applicable to the MACSQuant Analyzer. This function is not available to MACSQuantify Software installations on personal computers.

1) Click **Edit**, **Options, Software** and **Timers.**

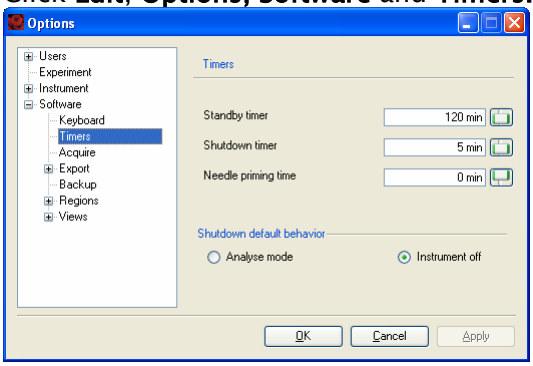


Figure 6.46 Changing the default timer settings.

2) Use the slider bar or text field to change the timers for the:
Standby timer: instrument goes into standby after the stipulated time of inactivity.
Shutdown timer: instrument performs a controlled shutdown after the stipulated time of inactivity.
Needle priming timer: instrument performs an automated needle priming at stipulated intervals.

3) **Shutdown** can be defined as **Instrument off** (default) or **Analyse mode** (the instrument switches to **Analyse mode** and does not power-off).

4) Click **Apply** to implement changes. Click **OK** close the window.

5) Click **Edit**, **Options, Software** and **Timers.**

To assign default settings for Acquire:

Note: Feature only applicable to the MACSQuant Analyzer. This function is not available to MACSQuantify Software installations on personal computers.

1) Click **Edit**, **Options, Software** and **Acquire.**

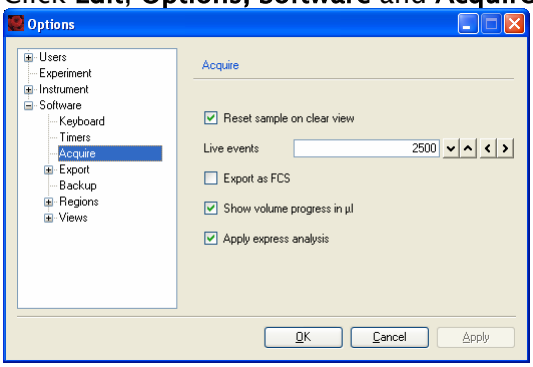


Figure 6.47 Changing the default Acquire settings.

2) Use the check boxes to activate a feature during data acquisition (live mode):
Reset sample on clear view: plot or chart is automatically cleared after data acquisition, i.e. it is not necessary to click the Clear icon (

).
Export as FCS: acquired data is always exported as FCS.
Show volume progress in µL: during sample uptake and processing the MACSQuant Analyzer will continually report changes to the sample volume in the Status Bar.
Apply express analysis: The option to perform analysis in Express mode is always given.

3) Click **Apply** to implement changes. Click **OK** close the window.

To assign default settings for Statistic Export:

1) Click **Edit**, **Options**, **Software** and **Export** and **Statistic**.

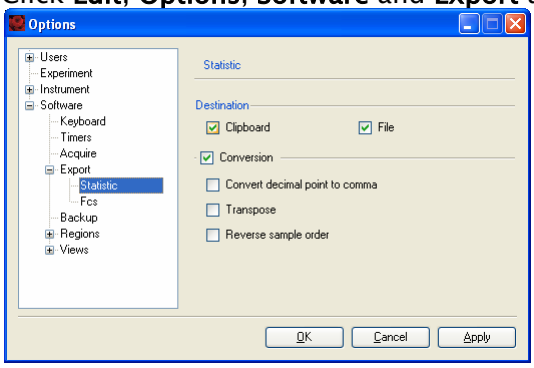


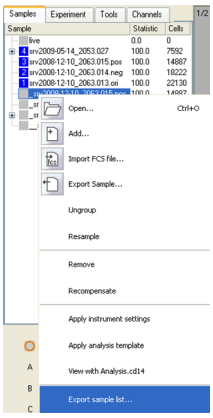
Figure 6.48 Changing the default Statistic export settings.

2) Use the check boxes to activate a feature:
Clipboard: plots and data tables can be copied to Windows Clipboard.
File: plots and data table can be exported to another file.
Conversion: activate the following conversion is options for file export:

a. **Convert decimal point to comma**: the decimal point is converted to a comma.

b. **Transpose**: Data is exported as a table comprising rows and columns. When Transpose is checked the table format is inverted i.e. columns become rows and rows become columns.

c. **Reverse sample order**: When exporting sample lists, samples can be saved to an excel table in descending or ascending (“reverse”) order. In the following example, exporting the sample list with **Reverse** checked will save the sample list in ascending order i.e. from 1 to 4.



3) Click Apply to implement changes. Click OK close the window.

To assign default settings for FCS Export:

1) Click **Edit**, **Options**, **Software** and **Export** and **Fcs**.

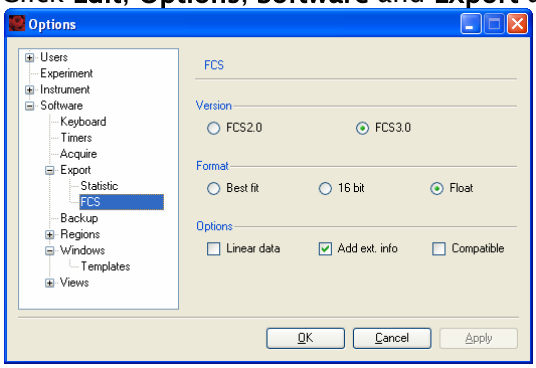


Figure 6.49 Changing the default Fcs export settings.

2) Use the check boxes and/or radio buttons to activate/deactivate a feature:
Version: the default file export format can be either FCS2.0 or FCS3.0.
Format: Data can be saved as Best fit, 16 Bit or Float. The 16 bit format is compatible with most data handling software.
Options:
Linear – All data will be saved in linear format i.e. without logarithmic manipulation.
Add ext. info – Information about the file format, time/data and file type is added to a text header of the data file. As this information varies according to the size of the data file, the text header may also vary in size. Some non-Miltenyi Biotec flow cytometry data handling software are unable to work with files which have a larger info-text header. It is therefore recommended to disable this function by default.
Compatible – Export data for use with other flow cytometry analysis software, for example, FlowJo. In actual fact data is exported as Float and Linear data.

3) Click **Apply** to implement changes. Click **OK** close the window.

To assign default settings for Backup:

1) Click **Edit**, **Options**, **Software** and **Backup**.

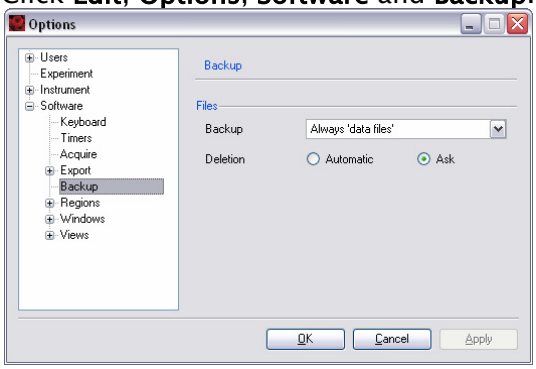


Figure 6.50 Changing the default file backup settings.

2) Use the dropdown list and/or radio buttons to activate/deactivate a feature: **Files Backup**:

   a. **always ask** – before performing a backup the user is prompted to verify which file types are to be backed-up.

   b. **always 'all files'** – all files are always backed-up, there is no user prompt to verify this procedure.

   c. **always 'data files'** – only data files are backed-up, there is no user prompt to verify this procedure.

3) **Files Deletion**:

   d. **automatic** – automatically overwrite or delete files during backup.

   e. **ask** – the user is prompted to verify deletion or overwriting of files during backup.

4) Click Apply to implement changes. Click OK to close the window.

To assign default drag and drop properties for Regions:

1) Click **Edit**, **Options**, **Software** and **Regions**.

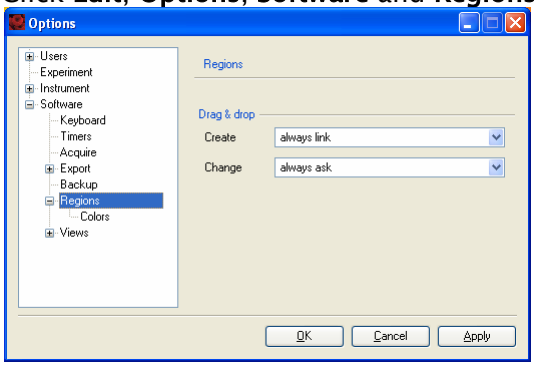


Figure 6.51 Changing the default settings for drag & drop of regions.

2) Use the dropdown lists to activate/deactivate a feature:
Create: when creating a new region
Change: when changing a region

3) Click Apply to implement changes. Click OK close the window.

To assign default color properties for Regions:

1) Click **Edit**, **Options**, **Software**, **Regions** and **Colors**.

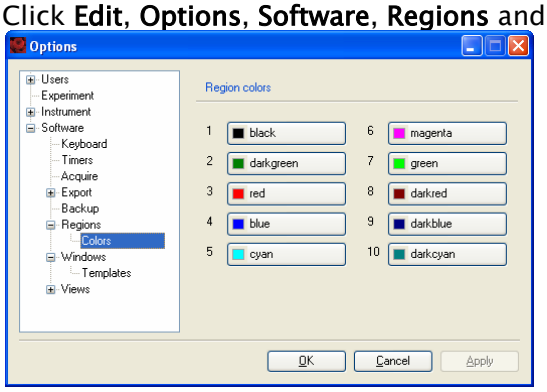
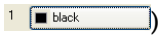


Figure 6.52 Changing the default color settings for regions.

2) Click on the color panel button adjacent to the region that should be changed (e.g., 

).

Note: Regions are assigned numbered in ascending order, i.e., the first region to be drawn is assigned the value 1; the second is assigned the value 2 etc.

3) Select a new color. Click **OK**.

4) Click **Apply** to implement changes. Click **OK** close the window.

To assign default properties for Windows and Window Templates:

1) Click **Edit**, **Options**, **Software**, **Windows**.

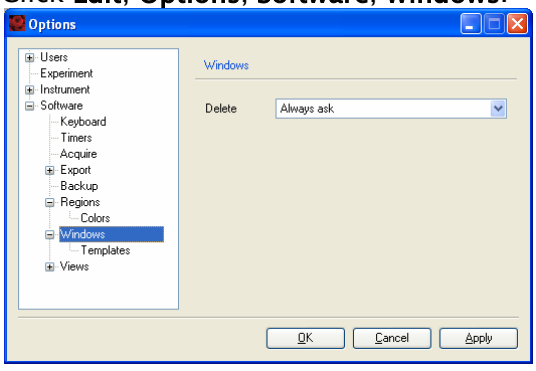


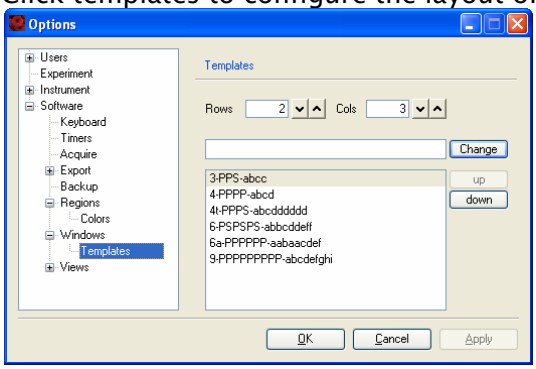
Figure 6.53 Changing the default color settings for windows.

2) It is possible to configure a "warning prompt" when users click  in order to close an analysis window.

- **Never ask**: the window will immediately close when  is clicked.

- **Always ask**: the user will be prompted to "Close the current window" when  is clicked.

3) Click templates to configure the layout of window templates:

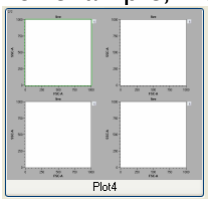


Plots types are assigned the following nomenclature:
P=Dot plot; D=Density plot; H=Histogram; T=Text; S=Statistic; N=None (blank).

The total number of plots in a plot layout is given by a number, from 1 to 16.

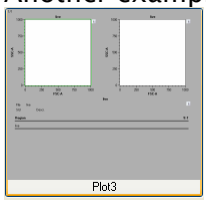
The position of a plot in the plot layout is assigned by letters: abcdefg…etc. where position A is at the top-left hand corner of the plot window, and the last letter used is at the bottom right-hand corner of the plot window. Letters can also be reused, e.g. aabb; this allows the user to assign a plot type over two or more positions.

For example, Plot4 can be described as 4-PPPP-abcd:



That is, 4 plots will be assigned to the total 'workspace'. Each plot will be dot plots (P). abcd denotes that each dotplot will be placed in each corner of the 'workspace'.

Another example: Plot3 can be described as 3-PPS-abcc:



That is, 3 plots will be assigned in total. Of which two will be dot plots (PP) and one will be a Statistical table (S). The dot plots will occupy the upper-left and upper-right corners of the 'workspace' (ab). The Statistics table will occupy the entire lower-half of the 'workspace' (cc).

Note: Hyphens (-) are used to separate the conditions used to describe plot layouts (e.g. "3-PPS-abcc"). Only hyphens may be used.

4) Configure the dot plots as necessary. Click **Apply** to implement changes. Click **OK** to close the window.

To assign default properties for displayed plots, histograms and tables – "Views":

1) Click **Edit**, **Options**, **Software**, and **Views**.

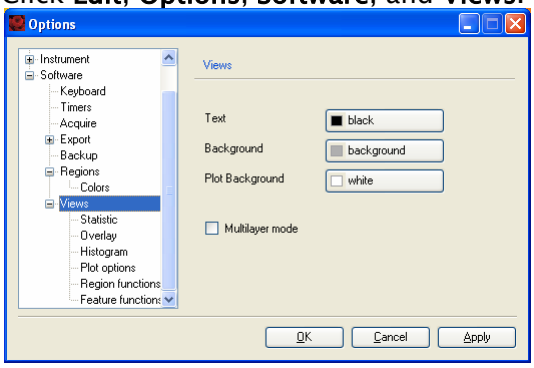


Figure 6.54 Changing the default "Views" settings for plots, histograms and tables

2) Select the required view category:

a. **Statistic** – click checkbox to display the header for the statistic table.

b. **Overlay** – the user is prompted to verify deletion or overwriting of files during backup.

c. **Histogram** – use dropdown menus to select default values for displaying histograms: Normalization, Smoothing and Mode.

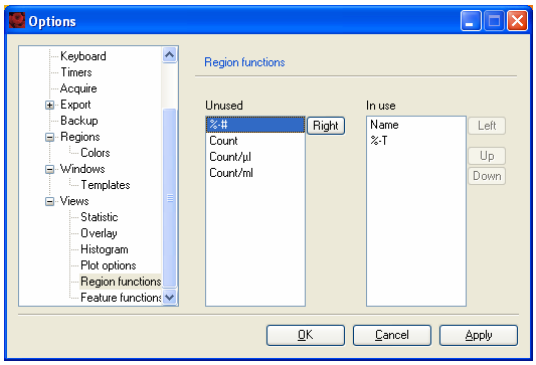
d. **Plot options:**

i. **Data**:
All – displays all acquired events by default.
Percentile – displays a percentile value of the total acquired events by default, namely, 1%, 2%, 5%, 10%, 25%, 50%.
Fixed number – displays a stipulated number of acquired events by default.

ii. **Axes**:
X-Axis – displays the selected axis scale by default.
Y-Axis – displays the selected axis scale by default.

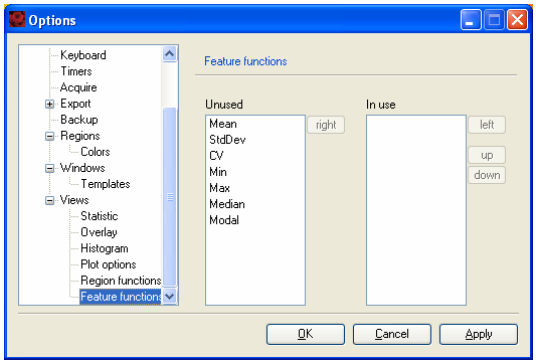
Note: It is recommended to use the default setting "**As required**". When performing data analysis users can modify the axis scale as required.

e. **Region functions** – select and highlight a function and using the Left and Right buttons move the region into the desired category: **Unused** or **In use**.



- **Name**: The name axis name.
- **Count:** The actual total acquired events or count.
- **Count/µL:** Number of acquired events per microliter.
- **Count/mL:** Number of acquired events per milliliter.

f. **Feature functions** – select and highlight a feature function and using the Left and Right buttons move the feature into the desired category: **Unused** or **In use**.



3) After making the necessary modifications, click **Apply** to implement changes.

4) Click **OK** close the window.

6.12 Data analysis in Custom mode

Acquired data are displayed and analyzed by an analysis window. Depending on which "**New plot window"** template is applied by the user, analysis windows may contain dot plots, density plots, histograms, statistic and text tables. Several analysis windows can be opened at one time. These can be of several experiments or of a single experiment with a complex gating strategy. Gating strategies can be created during sample acquisition (Live) and saved for future use or can be created post-acquisition.
For background information concerning "gating" or defining regions of interest refer to section 2.2.8.

6.12.1 Creating a new analysis template or analysis window

Analysis templates consist of a plot template (new analysis window) and a gating strategy. Analysis templates can be created post-acquisition or during acquisition in "Live mode".

To create a useful analysis template, whether in Live mode or post-acquisition, the **Experiment settings** must have been correctly defined. See section 6.3.6 for more details.

An **analysis window** can be saved as an analysis template. Click the **New analysis window** icon ( ) or use the file menu option: **Window** and **New analysis window** to create a new analysis window.

To create a new plot window:

1) Click the icon or use the file menu option **Window** and **New analysis window**.

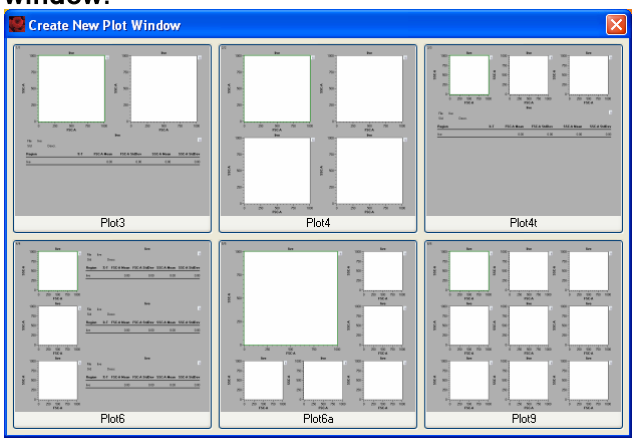


Figure 6.55 Available analysis templates are displayed by the "Create New Plot Window".

2) Click on the required analysis template.

3) The analysis template will open as shown below.

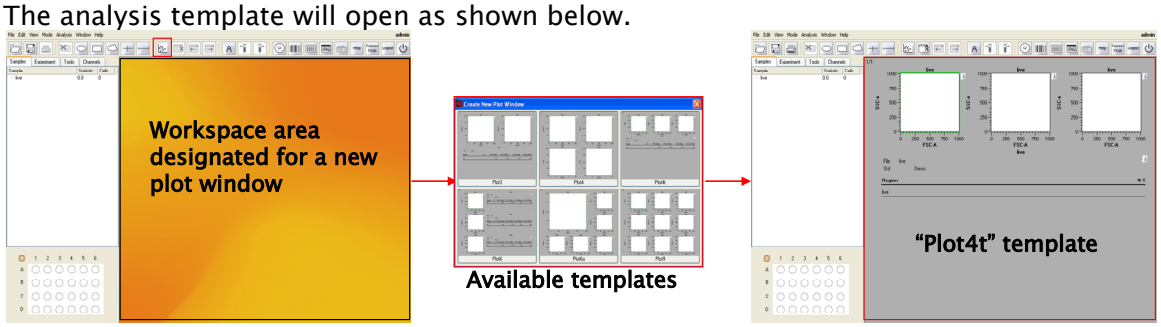


Figure 6.56 Selecting a new analysis template

Note: Multiple analysis windows can be opened. These can be of single or multiple experiments.

Note: If multiple analysis windows are open, use the top menu bar icons to display the previous and next analysis window.

6.12.2 Choosing a display format for plots and histograms

The layout of an analysis template is predefined as follows:

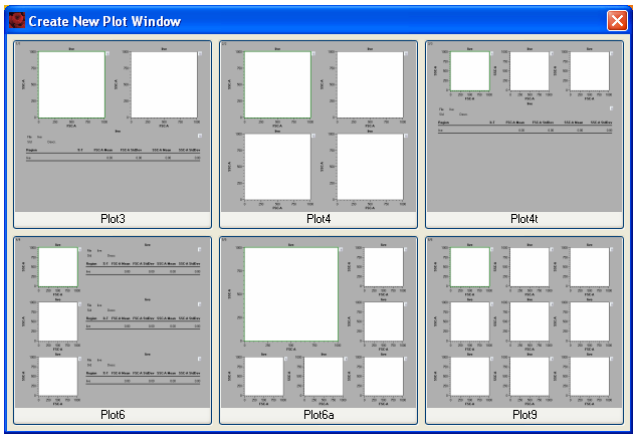


Figure 6.57 Six analysis templates are available to choose from.

Six analysis templates are available to choose from. The size and layout of the dot plot windows are predefined; however, users can change the properties of each individual plot using the icon.

Note: To modify these settings during live data acquisition the MACSQuantify Software must NOT be in analysis mode, i.e. . To modify settings on post-acquired data (data opened from a file), the MACSQuantify Software must NOT be in analysis mode, i.e. .

Custom mode users can select a display format for data and text as follows:

1) Click on the icon, , located beside the dot plot, histogram or text table. A popup menu will appear.

Figure 6.58 Five display formats may be chosen: Dot plot, Density plot, Histogram, Statistic or Text.

2) Select the desired display from the popup menu.

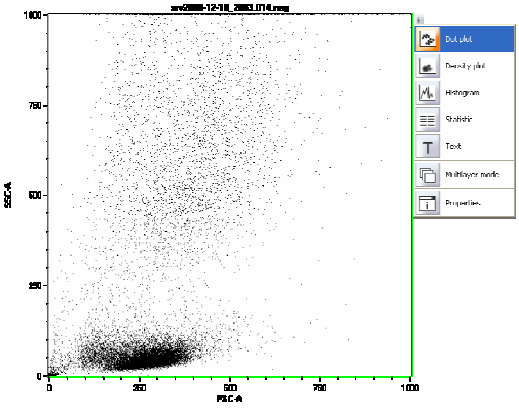


Figure 6.59 Dot plot format was chosen to display the opened dataset.

3) Optional: Click |  | to save changes to the Workspace.

6.12.3 Changing the properties of a plot, histogram or text table

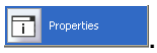
Note: The MACSQuantify Software can display data in four discrete formats: Dot plot, density plot, histogram and statistic. In addition text may also be displayed in a textbox. Refer to section 2.2.7 for an explanation of these formats.

Note: For information about file handling in Custom mode (e.g. opening and saving data files) refer to section 6.9.1.

Custom mode users can change the properties of a dot plot, density plot, histogram, and statistic or text box as follows:

1) Click on the icon,  , located beside the dot plot, histogram or text table. A popup menu will appear.

Figure 6.60  **– Popup menu.**

2) Click Properties, 

. The properties window will appear.

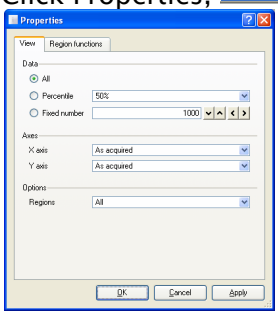


Figure 6.61 Properties window.

3) Select the desired property and modify accordingly. For example, to change the axes of a dot plot use the **Axes** dropdown menu.

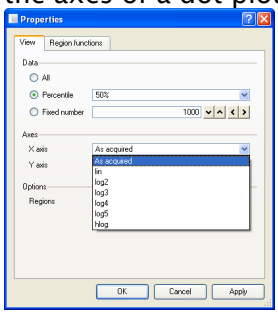


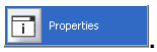
Figure 6.62 Changing the axis properties of a dot plot.

> **Note:** The Properties window for **Dot plots** and **Density plots** is identical. The Properties window for **Histogram**, **Text** and **Statistic** are different. Refer to the following sections for an overview of the settings available for each chart type:
> Plots: "Overview of the "Properties" settings for Dot plots and Density plots:".
> Histograms: "Overview of the "Properties" settings for Histograms:"
> Statistics: "Overview of the Properties settings for the Statistic option:"
> Text: "Overview of the "Properties" settings for Text:"

1) Click **Apply** to apply changes.
2) Click **Ok** to apply changes and close the **Properties** window.

Overview of the "Properties" settings for Dot plots and Density plots:

A variety of display settings for dot plots, histograms and density plots can be modified using the **Properties** option from the "  " dropdown menu.

1) Click the icon,  , located adjacent to the dot plot, histogram or density plot.
2) Click Properties, 

. The properties window will appear.

> **Note:** Refer to Table 6.8 below for an overview of the various display **Properties** that may be modified for dot plots and density plots using the MACSQuantify Software.

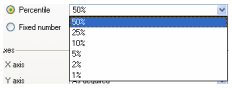
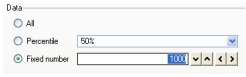
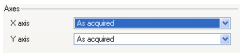
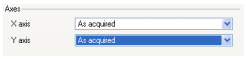
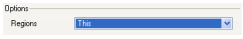
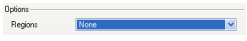
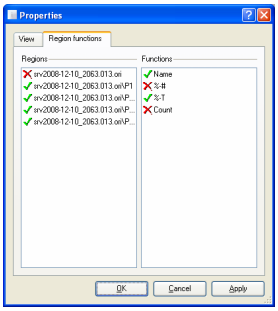
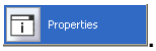
Tab option	Category	Property	Associated screenshot	Description
View	Data	All		All events are displayed on a dot plot or density plot.
		Percentile		50%, 25%, 5%, 2% or 1% percentile values of the total events can be displayed on a dot plot or density plot. This is useful when too many events have been acquired and the displayed plot/histogram is saturated.
		Fixed number		A fixed number of events can be displayed on a dot plot or density plot. Numbers can be entered directly into the field or by using the arrows.
	Axes	X axis		The y- and x-axes scales of a dot plot and density plot, or the x-axis scale of a histogram can be configured as follows: As required – automatically configured lin – linear scale log2-5 – logarithmic scales hlog – hyperlog scaling
		Y axis		
	Options	All		All regions (“gates”) are shown on the chart.
		This		Only a select region (“gate”) is shown on the chart.
		None		No regions (“gates”) are shown on the chart.
Region functions		Regions		Regions (“gates”) can be displayed (✓) or hidden (✗) by selecting or deselecting a region using the left mouse button or by touching the display with your fingertip.
		Functions		Functions for a region can be displayed (✓) or hidden (✗) by selecting or deselecting the function using the left mouse button or by touching the display with your fingertip. The following functions can be changed for plots and histograms: Name: Region name e.g. “P1” %-#: Events in selected region are displayed as a percentage of the total number events in the current gating strategy. %-T: Events in selected region are displayed as a percentage of the total number of acquired events. Count: The actual number of events occurring in the selected region is displayed.

Table 6.8 An overview of the “Properties” settings for dot plots and density plots.

Overview of the "Properties" settings for Histograms:

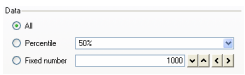
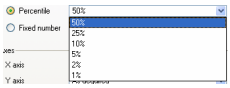
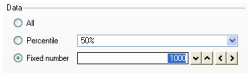
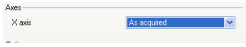
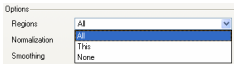
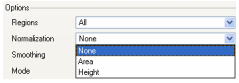
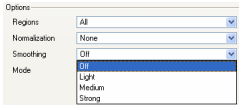
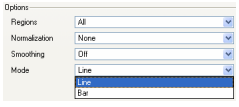
A variety of display settings for Histograms can be modified using the **Properties** option from the "  " dropdown menu.

1) Click the icon,  , located adjacent to the histogram.

2) Click Properties, 

. The properties window will appear.

Note: Refer to Table 6.9 below for an overview of the various display **Properties** that may be modified for histograms using the MACSQuantify Software.

Tab option	Category	Property	Associated screenshot	Description
View	Data	All		All events are displayed on the histogram.
		Percentile		50%, 25%, 5%, 2% or 1% percentile values of the total events can be displayed on the histogram. This is useful when too many events have been acquired and the displayed plot/histogram is saturated.
		Fixed number		A fixed number of events can be displayed on the histogram. Numbers can be entered directly into the field or by using the arrows.
	Axes	X axis		The x-axis scale of a histogram can be configured as follows: As required – automatically configured lin – linear scale log2-5 – logarithmic scales hlog – biexponential scale
	Options	Regions		No regions ("None"), all regions ("All") or only the selected region ("This") can be shown on the histogram.
		Normalization		The histogram graphically summarizes the distribution of a univariate data set. Data can be normalized by Area (integral of total area under the curve) or by Height.
		Smoothing		Algorithms can be used to smooth the histogram. Light, medium or strong smoothing is available.
		Mode		Histograms can be display as a line chart or bar chart. The latter is used to a greater degree.

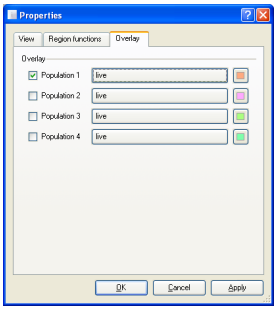
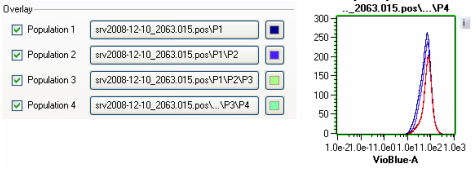
Region functions		Regions		The settings for regions and functions are identical to those for dot plots.
		Functions		
Overlay		Population		Several regions (gated “cell populations”) can be displayed on a single histogram. In the following example four regions (P1, P2, P3, and P4) were color-coded and displayed. 

Table 6.9 An overview of the “Properties” settings for histograms.

Overview of the Properties settings for the Statistic option:

A **Statistic** text box is composed of a **Header** and a **Table**.

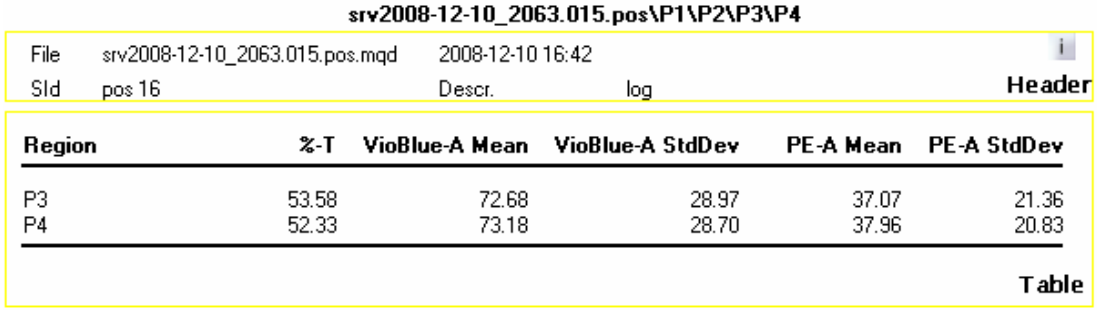


Figure 6.63 A header and a table comprise a statistic text box.

A variety of display settings for the **Statistic** option can be modified using the **Properties** option from the “ ” dropdown menu.

1) Click the icon, , located adjacent to the **Statistic** textbox.
2) Click Properties, . The properties window will appear.

> **Note:** Refer to Table 6.10 below for an overview of the various display **Properties** that may be modified for the Statistic option using MACSQuantify Software.

To change the contents of the statistic text box refer to **Error! Reference source not found.** below.

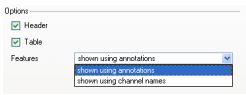
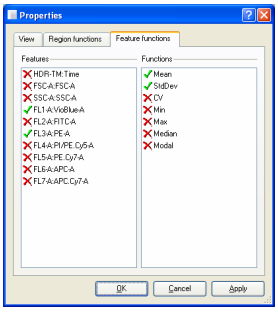
Tab option	Category	Property	Associated screenshot	Description
View	Options	Header		Check the Header box to include this information in the text box.
		Table		Check the Table box to include this information in the text box. Column headers (titles) of the table can be displayed in two formats: “shown using annotations” and “shown using channel names”. Annotations are defined by the user. For example in example Error! Reference source not found., channel “FL1-A” was annotated as “VioBlue-A”.
Region functions		Regions		The settings for regions and functions are identical to those for dot plots.
		Functions		
Feature function		Features		The recorded time, scatter, and fluorescence data acquired from each channel may be displayed using the Features setting. The data shown will be dependent on the selected regions (see Region functions). Features can be displayed (✓) or hidden (✗) by selecting or deselecting a feature using the left mouse button or by touching the display with your fingertip.
		Functions		Statistics (or “**Functions**”) for each selected **Feature** can be displayed (✓) or hidden (✗) by selecting or deselecting a Function using the left mouse button or by touching the display with your fingertip. The following statistical functions may be displayed: Mean; StdDev (standard deviation); CV (coefficient of variation); Min (minimum); Max (maximum); Median and Modal values.

Table 6.10 An overview of the “Properties” settings for the Statistic option.

Overview of the “Properties” settings for Text:

A variety of display settings for the Text option can be modified using the **Properties** option from the “ ” dropdown menu.

1) Click the icon, , located adjacent to the Text box.

2) Click Properties, . The properties window will appear.

> **Note:** Refer to Table 6.11 below for an overview of the various display **Properties** that may be modified for Text boxes using the MACSQuantify Software.

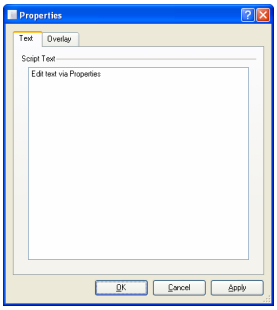
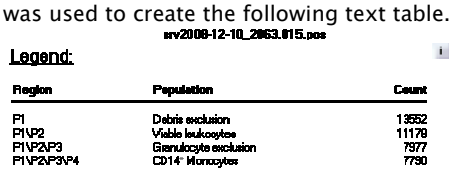
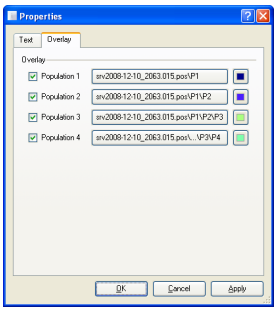
Tab option	Category	Property	Associated screenshot	Description
Text	Script Text			Free text or a MACSQuantify Software script can be entered into the "Script Text" field. Scripts are based on HTML (hypertext mark-up language) and can be written to automatically display statistics about each region or gate. Specific regions can be removed from or added to text table by using the Overlay tab. A script was used to create the following text table.  Scripts are provided by MACSQuant Analyzer specialist. Please contact your Miltenyi Biotec advisor for further information.
Overlay	Overlay	Population		If a MACSQuantify Script has been used (Text tab) it possible to add or remove regions (populations) by using the Population check box. In the following example all four regions (P1, P2, P3, P4) of a gating strategy were checked and therefore displayed. 

Table 6.11 An overview of the "Properties" settings for Text boxes.

6.13 Working with regions or "gates"

For background information concerning drawing regions with MACSQuantify Software refer to section 2.2.8.

6.13.1 Drawing regions

Note: In the following example the **IL-17 Secretion Assay – Cell Enrichment and Detection Kit** (order no: 130-094-542) was used for the analysis of viable human IL-17 secreting leukocytes from PBMCs.

Enriched human IL-17 secreting cells were labeled as follows:

- IL-17 Detection Antibody – PE conjugated fluorochrome
- CD4 marker – FITC conjugated fluorochrome
- CD154 marker – APC conjugated fluorochrome

Note: For background information about drawing regions (gating) with MACSQuantify Software refer to section 2.2.8.

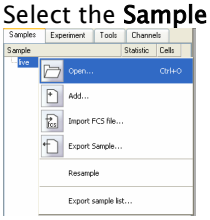
To create/draw a region:

1) Open a data file or simply draw a region around events that are being acquired by the MACSQuant Analyzer in real-time.

- Click **File, Open** and highlight the data file(s) to be analyzed. Click **Open**.

  Alternatively;

- Select the **Sample tab** and right-click **Open** in the sample window:



  Highlight the data file(s) to be analyzed. Click **Open**.

2) Click  to open an analysis window. Select the desired plot layout.
   In this case a **Plot4** layout was chosen.

3) Select the plot of interest. A green border indicates the chosen plot.

4) Double-click on the opened data file (this is not necessary when acquiring data in real-time).

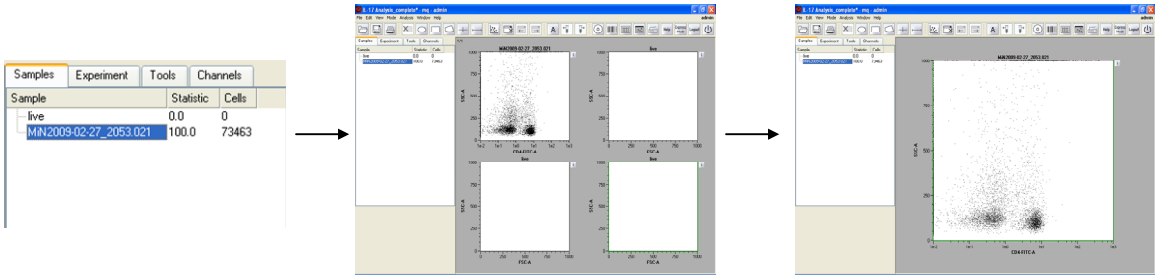


Figure 6.64 Left: The data file MN2009-02-27_2053.005 was opened in the pre-selected plot window (middle). Right: Double-clicking on the middle plot window enlarges the plot to cover the entire analysis window.

5) Use the icons to select a geometrical shape for gating:

Note:  or "Interval" can be only used for **Histogram** analysis. Interval is a marker or region that can be drawn on histograms in order to calculate statistics for that particular region.

6) Axis: forward-scatter versus side-scatter.
   A region was drawn (P1) using the polygon tool () to exclude unwanted

debris and select for lymphocytes.

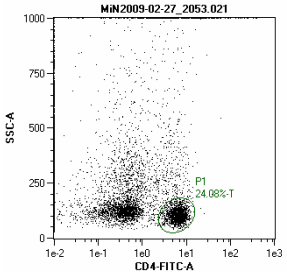


Figure 6.65 A polygon region (P1) was drawn to exclude unwanted debris and select for CD4+ lymphocytes

7) Axis: Anti-IL-17-PE versus PI/PE-Cy5.

A polygon region was drawn (P2) using the polygon tool ( ) to exclude unwanted dead cells.

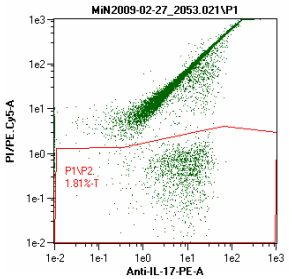
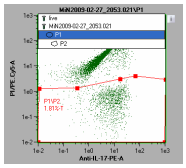


Figure 6.66 A polygon region (P1/P2) was drawn to exclude unwanted dead cells

8) The region P2 was only displayed on the third plot

Note: Click on the plot header to select a region to display.



Click to switch on/off the Multilayer mode.

Switch on Multilayer mode to view all regions on a plot.

Switch off the Multilayer mode to only view the region displayed on the plot header.

---

9) Axis: Anti-IL-17-PE versus Anti-CD4-FITC.

A rectangle region was drawn (P3) using the rectangle tool ( ) to select for IL-

17+ CD4-viable lymphocytes.

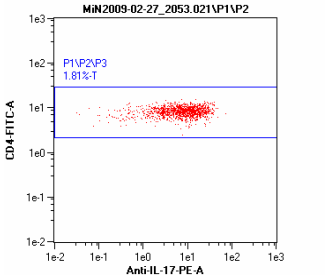
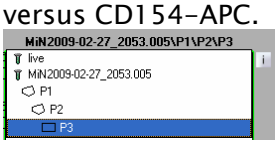


Figure 6.67 A rectangle region (P1/P2/P3) was drawn to select for activated IL-17 CD4+ lymphocytes.

10) Axis: Anti-IL-17-PE versus Anti-CD154-APC.

The region P3 (gate i.e. P1/P2/P3) was displayed using the axis Anti-IL-17-PE versus CD154-APC.



A final population of enriched activated IL-17 secreting CD4+ T cells are shown.

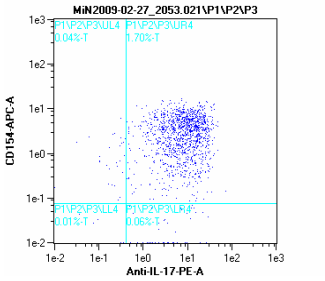


Figure 6.68 The region P1/P2/P3 was displayed using the axis Anti-IL-17-PE versus CD154-APC.

11) Click ⊞ to expand the gating strategy in the **Samples** menu i.e.

⊞ MiN2009-02-27_2053.005 100.0 229334

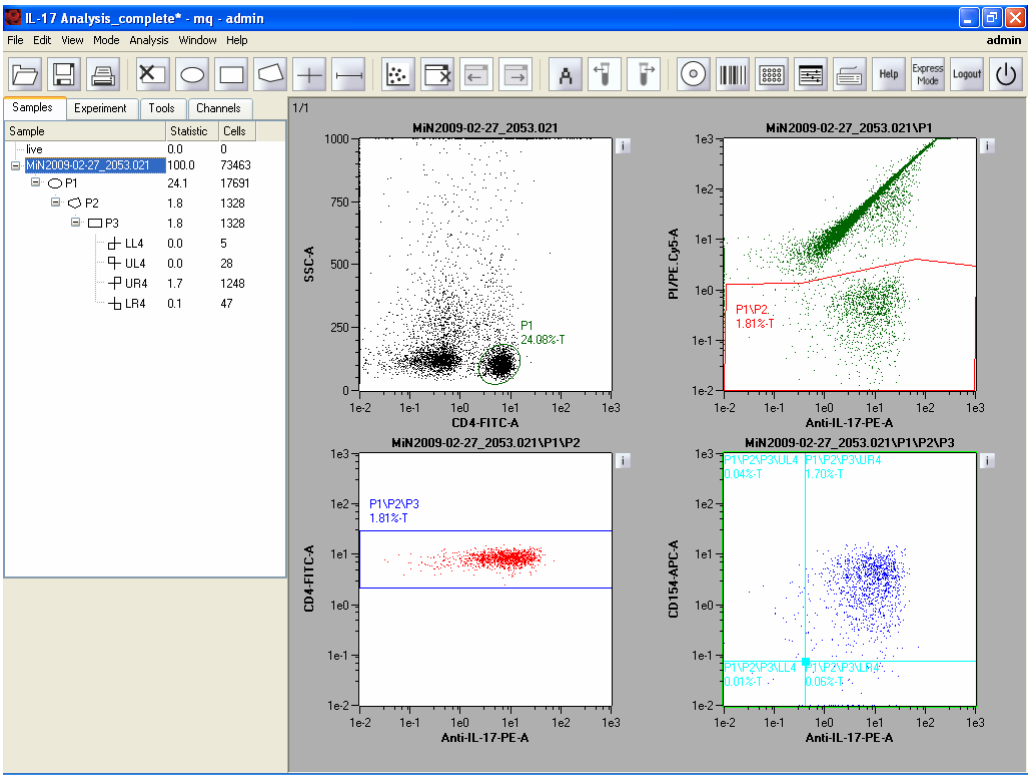
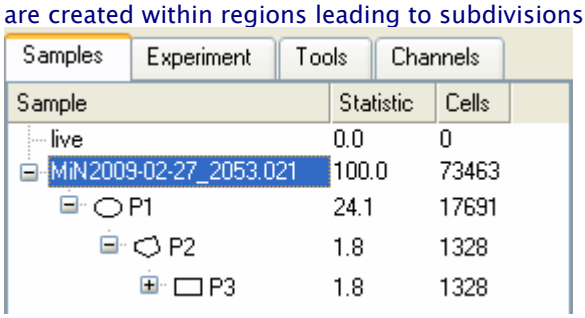


Figure 6.69 An overview of the entire gating strategy.

Note: The regions in this "gating" strategy do not have the same hierarchy. Regions are created within regions leading to subdivisions of gates/regions–:



Refer to section 6.13.2 for information on creating other gating strategies.

12) To save the "gating strategy" as an Analysis template:

a. Ensure that the MACSQuantify Software is in Analysis mode: 

b. Click  and 

c. Choose the file location: **Public**, **Private** or **External**.

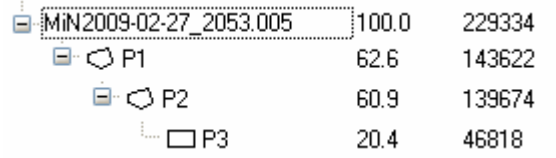
d. Name the file. Click **Save**.

6.13.2 Gating stratgies

Note: For background information about drawing regions (gating) with MACSQuantify Software refer to section 2.2.8.

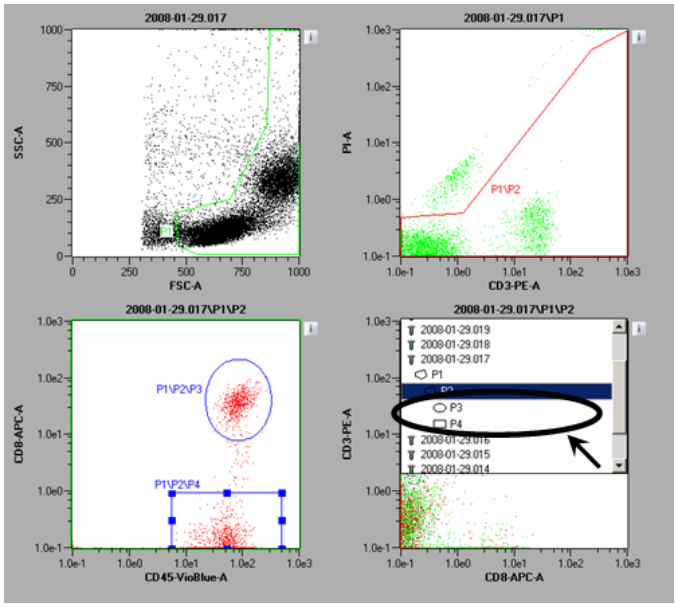
Classical hierarchical gating

In section 6.13.1 an example of a hierarchical gating strategy is given. In this classical strategy 'gates' are created within 'gates' in order to identify sub-populations of cells.



Regions having the same hierarchy

It is also possible to create regions having the same hierarchy. Regions drawn in a single plot have the same hierarchy. In the example below, regions P3 and P4 (bottom left) were both defined within Region P2 (top right) and therefore have the same hierarchy.



"NOT" gates

So called NOT gates are used to eliminate a cell population from analysis. To create a NOT gate:

1) Draw a region or gate around the population to be excluded from analysis

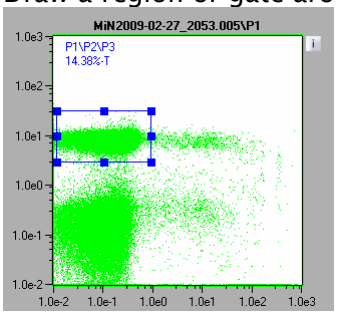
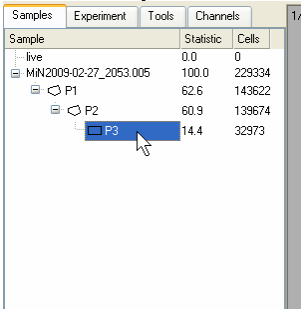


Figure 6.70 Region P3 was drawn using the Rectangle drawing tool

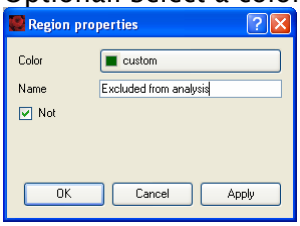
2) In the **Samples** menu, right-click on the region of interest, i.e. in this case P3



3) Select **Region properties** from the drop-down list.

4) Check the box **Not**. This region is now excluded from analysis.

Optional: Select a color and/or name the NOT gate as desired.

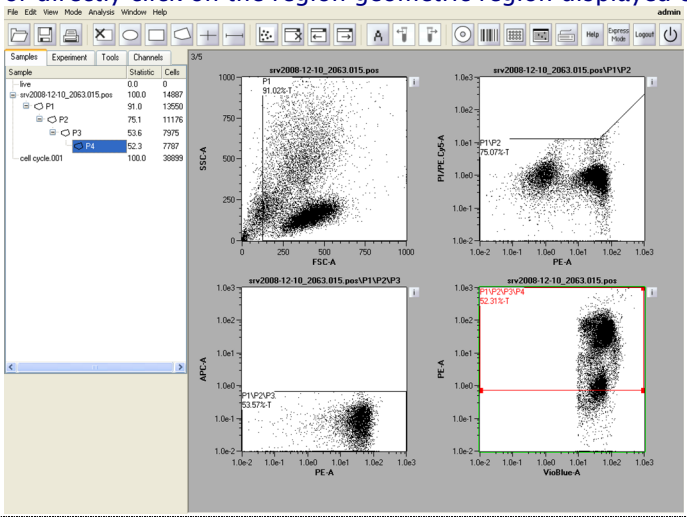


5) Click **Apply** and **OK**.

6.13.3 Changing the properties of regions

1) Click on the region of interest.

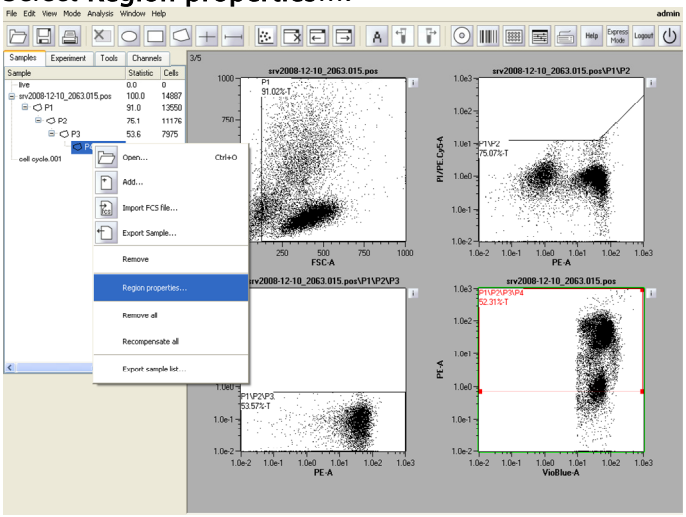
Note: To select a region, click on the Region name using the **Sample** menu (P4) or directly click on the region geometric region displayed on the plot:



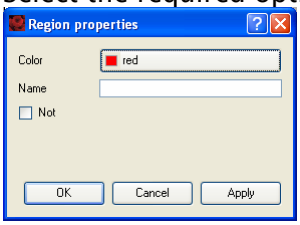
2) To change color, region name and/or define the region as NOT:

a. Right-click on the Region name displayed in the **Sample** menu.

b. Select **Region properties**….



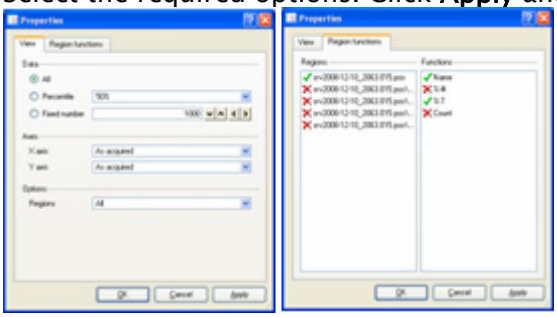
c. Select the required option(s). Click **Apply** and **OK**.



3) To change region functions:

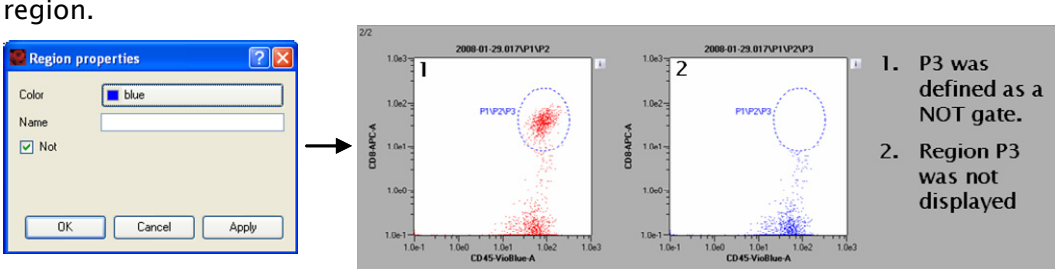
a. Click the  icon which is adjacent to the plot of interest.

b. Select the required options. Click **Apply** and **OK**.



Note: Refer to section 6.12.3 for an overview of the plot properties features.

4) In the example below, region P3 was displayed in blue and set as a **NOT** region.

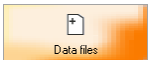


6.13.4 Post-acquisition data analysis

Note: For an overview of data handling (e.g. opening, saving and importing files) with the MACSQuantify Software refer to section 6.9.

To open saved data files:

1) Click **File** and **Open** or click the icon .

2) Click the **Data files** tab: 

3) Choose the desired data file(s) from a **Public**, **Private** or **External** source.

Note: Multiple files can be opened at once.

4) Click open.

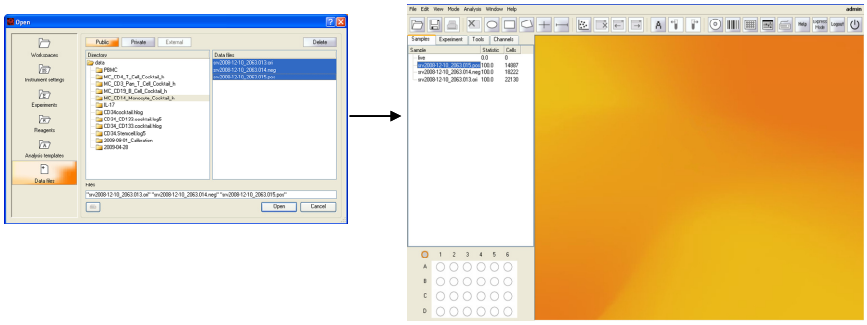


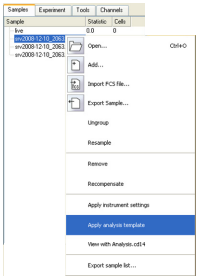
Figure 6.71 Three MACSQuantify Software data files were opened from a public (shared) location

Applying analysis templates to post-acquired data:

It is possible to apply or view an analysis template that was used in a previous experiment, i.e. the gating strategy and corresponding plots that are associated with acquired data files can be reapplied and viewed.

To apply an analysis template associated with a data file:

1) Open the desired data file(s).
2) Click on the **Sample** tab menu.
3) Right-click on the data file and select **Apply analysis template**.



4) The data file and corresponding analysis template will be loaded in Analysis mode ( ).

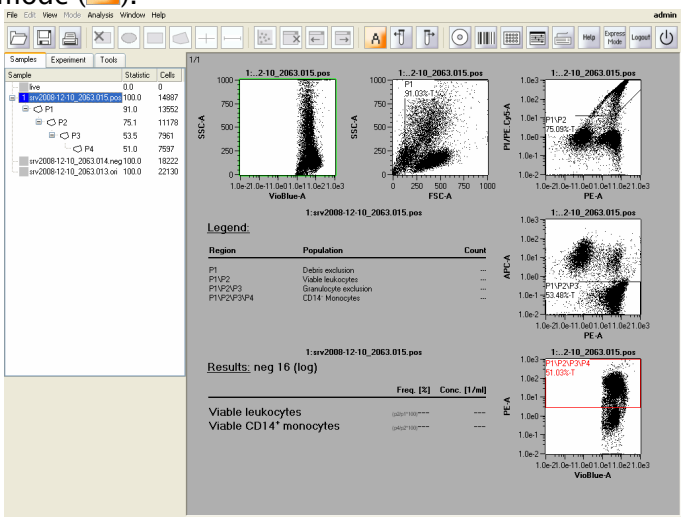


Figure 6.72 Application of an analysis template for the file srv2008-12-10_2063.015.pos. The template shows a gating strategy for analysis of CD14+ monocytes.

5) To **View** analyzed results of the previously defined analysis, right-click on the data file and select **View with analysis** *<name of analysis>*.

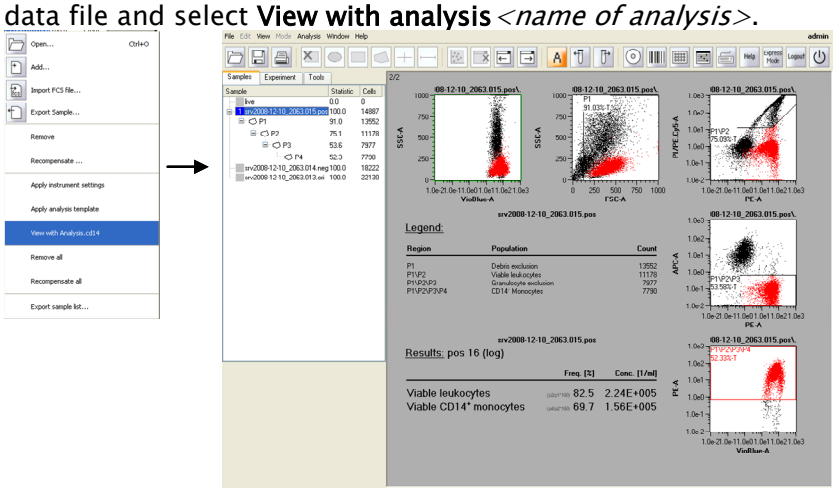


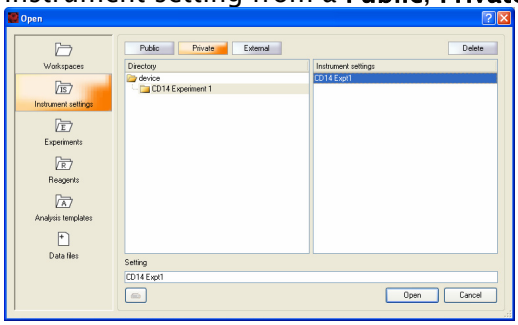
Figure 6.73 Viewing results of a CD14 analysis. CD14+ monocytes were analyzed using an appropriate experiment design and gating strategy using the MACSQuant Analyzer and MACSQuantify Software.

Applying previously saved instrument settings (PMT voltage and compensation settings):

It is possible to apply instrument settings associated with a data file.

To apply a previously saved instrument setting that is associated with a data file:

1) Click  and highlight the **Instrument settings** tab. Highlight the desired instrument setting from a **Public**, **Private** or **External** source.

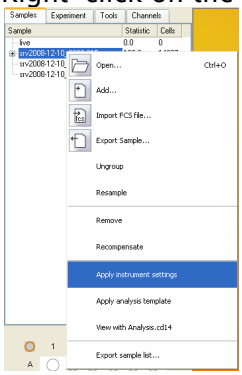


2) Click **Open**. The file settings will be loaded.

3) Open the desired data file(s). The files will be loaded and can be view from the **Sample** tab.

4) Click on the **Sample** tab menu.

5) Right-click on the data file and select **Apply instrument settings**.



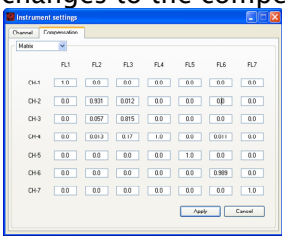
Recompensate post-acquired data:

It is possible to recompensate data that was already acquired. This is of benefit if data was acquired using incorrect instrument settings. The results can be reanalyzed using a different compensation matrix.

> **Note:** Refer to section 3.7 for an introduction to compensation and explanation of how instrument compensation is performed.

To recompensate saved data:

1) Open the desired data file(s). The files will be loaded and can be view from the **Sample** tab.
2) Click on the **Sample** tab menu.
3) Apply instrument settings/analysis template as instructed above [see Applying previously saved instrument settings (PMT voltage and compensation settings):].
4) Click  to view the instrument settings. Experienced users can modify the **Channel** and **Compensation** values if required. Click **Apply** to implement changes to the compensation matrix.



5) Right-click on the data file and select **Recompensate**.

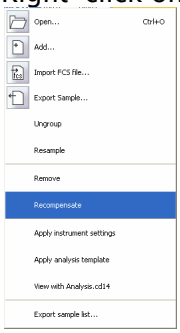
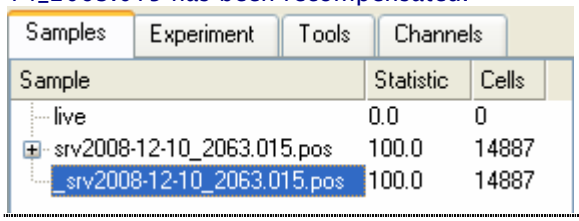


Figure 6.74 Recompensating a data file. It is also possible to recompensate all data files that have been opened and are shown in the "Samples" window.

Note: To recompensate all opened data files select **Recompensate all**.

Note: Recompensated files are prefixed with an underscore i.e. _srv2009-05-14_2063.015 has been recompensated.



6.13.5 Live gate

What is a live gate?

Only events within a live gate are acquired and saved by the MACSQuantify Software.

Note: All data outside of the gate will not be saved!

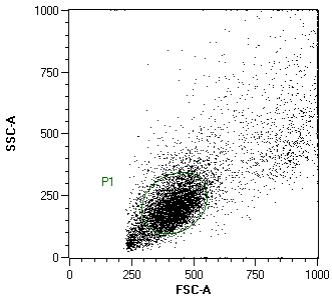


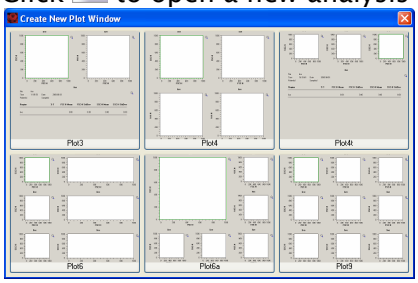
Figure 6.75 In this example, when using a live gate for P1, only events acquired within the region "P1" will be saved.

Live gates are useful when large datasets are being acquired. Since only data in a single gate are saved the subsequent data file is smaller. As a norm, however, it is recommended to acquire and save all data.

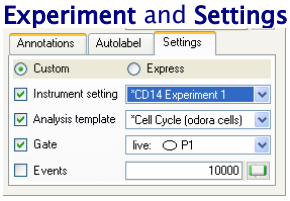
Live gating strategies can be saved for future use as **Analysis templates**.

To perform live gating:

1) Click  to open a new analysis window. Choose the required plot design.



2) Define the Experiment settings as required, e.g., uptake volume, sample name, flow rate, labeling strategy etc.

> **Note:** Ensure that the **Live gate** option for the appropriate gate is selected in the **Experiment** and **Settings** tab.
>
> 
>
>
> Live gate was chosen for region P1.
>
> **Note:** See section 6.3.6 for information about defining experiments; refer to section 6.7 for information about defining experiments with multisample processing.

3) Ensure that the correct instrument settings are loaded and that compensation is correctly performed.

> **Note:** See section 3.6 for information about performing instrument calibration; see section 3.7 for information about performing compensation.

4) Ensure that enough sample, reagents and buffers are provided.

5) Click on the **Start Measurement** button,  .
The MACSQuant Analyzer will commence sample uptake and measurement.

6) Draw regions on the plots as described in section 6.13.

> **Note:** Click  to delete all events displayed on a dot plot, i.e., to refresh the plot.

7) Save the **Analysis template** for future use.

6.13.6 Stop gate

What is a stop gate?

Unlike with a live gate, **all** data are acquired and saved by the so called **Stop gate**. However, a **Stop gate** used in combination with the **Events** option instructs the MACSQuantify Software to acquire data from the entire analysis window **until** a pre-

defined number of events are acquired within the **Stop gate** (i.e. a gate or region that is defined as the Stop gate).

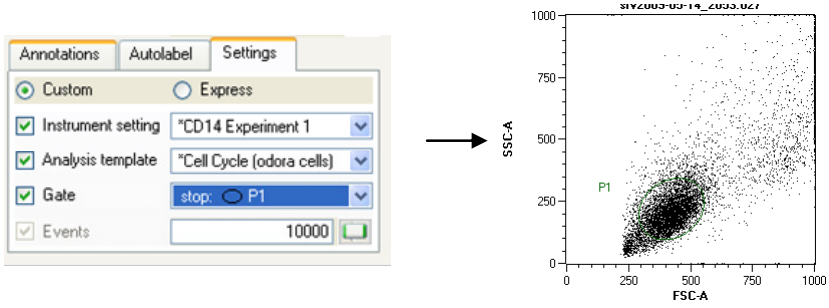
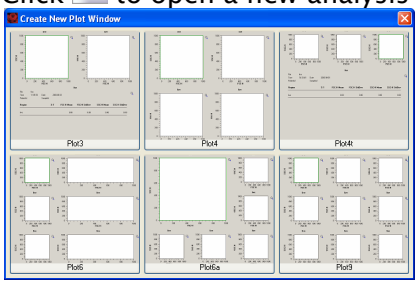


Figure 6.76 Region P1 was defined as a Stop Gate. When 1,000 events are acquired within region P1, data acquisition will automatically stop and all acquired data (i.e. the entire analysis window) will be saved.

Stop gating strategies can be saved for future use as **Analysis templates**.

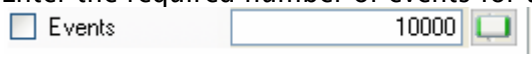
To perform stop gating:

1) Click  to open a new analysis window. Choose the required plot design.

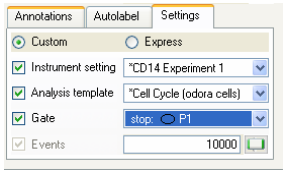


2) Define the Experiment settings as required, e.g., uptake volume, sample name, flow rate, labeling strategy etc.

3) Enter the required number of events for the associated Stop gate.



4) Check the **Gate** option and select the desired **Stop gate** from the drop-down list.



5) Ensure that the correct instrument settings are loaded and that compensation is correctly performed.

> **Note:** See section 3.6 for information about performing instrument calibration; see section 3.7 for information about performing compensation.

6) Ensure that enough sample, reagents and buffers are provided.

7) Click on the **Start Measurement** button,  .
The MACSQuant Analyzer will commence sample uptake and measurement.

8) Draw regions on the plots as described in section 6.13.

Note: Click clear to delete all events displayed on a dot plot, i.e., to refresh the plot.

9) Save the **Analysis template** for future use.

6.14 Grouping data post-acquisition

What is the benefit of sample grouping?

The maximum sample volume that can be acquired in a single step by the MACSQuant Analyzer is 450 µL. There are occasions when the sample size is of course greater; aliquots of the sample must therefore be spanned over two or more tubes.

By grouping these samples, the acquired data will be consolidated into a single file on the hard drive, which can also be analyzed in a single data file or analysis plot. This can be easily accomplished by grouping sample prior to data acquisition. Refer to section 6.8.3 for more details. If grouping was not performed prior to data acquisition, it is still possible to group samples post-acquisition.

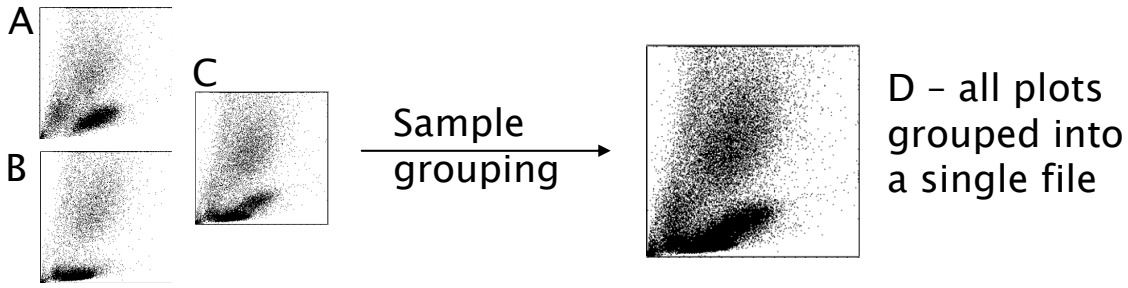


Figure 6.77 Schematic of the grouping process.

To group samples:

1) Click **File**, **Open** and navigate to the files that must be grouped. Highlight the files and click **Open**.

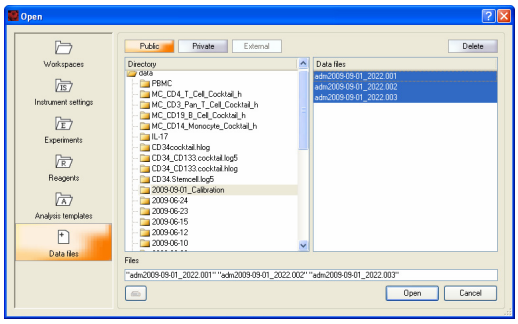


Figure 6.78 Three files were opened for grouping.

2) Using the **Samples** menu (Samples), highlight each file that must be grouped.

3) Right-click and select **Group**.

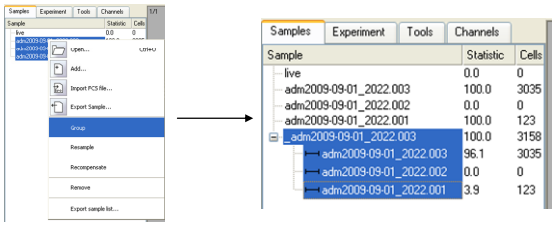


Figure 6.79 Left: Selecting three data files for grouping. Right: The resulting grouped files are highlighted.

6.15 Export sample list

It is possible to export the sample list to an excel table. The sample list table is a summary of all samples with corresponding statistics.

To export a sample list:

1) Sample on the Sample menu.

2) Highlight the sample files that must be exported. Right-click and select **Export sample list**.

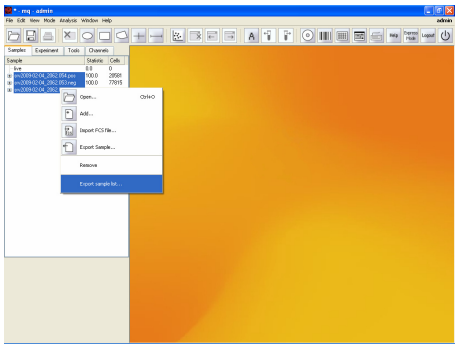
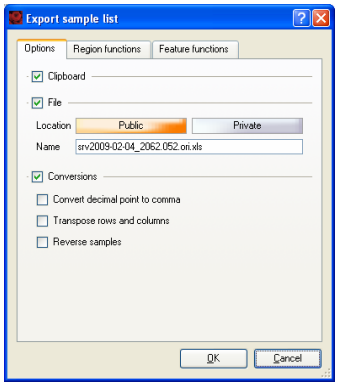


Figure 6.80 Three files were selected for export.

3) Configure the Export sample list box as follows:



- **Options**

  Clipboard : Check box to export data to the windows clipboard.

  File : Check box to export data to a Microsoft Excel file (xls).

  Location : Files can be saved to a Public or Private location.

  Name : If **File** is checked, enter the filename here, taking care not to delete the Excel file extension ".xls".

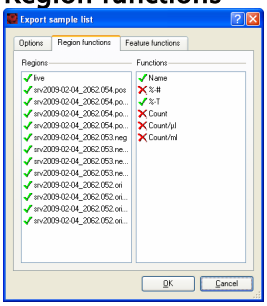
- Conversations – the respective boxes to:

a. Convert comma to point: in some languages numbers the decimal point is actually shown as a comma.

b. Transpose rows and columns: the columns and rows of the export table (e.g. Excel sheet) are inverted.

c. Reverse samples: the order of the samples (e.g. 1,2,3…etc) are reversed.

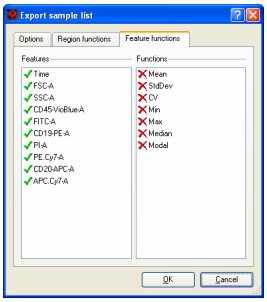
- **Region functions**



Select the gates/regions for export using the **Regions** box: ✓ = For export; ✗ =Not for export.

Similarly, use the **Functions** box to select/deselect region functions for export.

- **Feature functions**



In a similar manner, select/deselect **Features** (i.e. Channels) and **Functions** (i.e. statistics) for export.

4) After configuring the export options, click **OK**.
5) The file is exported to an Excel file and/or the clipboard.

> **Note:** If the relevant **Options** check-boxes have been selected, use Windows explorer to navigate to the exported Excel file or alternatively, paste the data into another windows application using the Edit – Paste command.

7 Shutdown of the MACSQuant Analyzer

The chapter outlines how a manual and automatic shutdown procedure is made.

7.1 Manual shutdown

If no further measurements are required, shut down the instrument as follows:

1) Click  to select ‘data analysis mode’ or ‘instrument off’.

   This automatically starts a washing protocol that lasts for approximately seven minutes, which includes an incubation of the washing solution in the fluidics followed by the flushing of the washing solution and replacement with the storage solution.

   Note: Data can be analyzed using the software interface even after the instrument has been shutdown using the analysis mode.

7.2 Automated shutdown

An automatic shutdown procedure can be activated using the MACSQuantify Software. This may be used to prevent the nonessential illumination of lasers (which shortens diode lifespan) or to guard against accidentally leaving the instrument on overnight or over the weekend.

Configuring automatic shutdown:

Note: Configuration of the automatic shutdown can only be performed by Custom users and administrators.

1) Click **Edit**, **Options...**, **Software** and **Timers**.

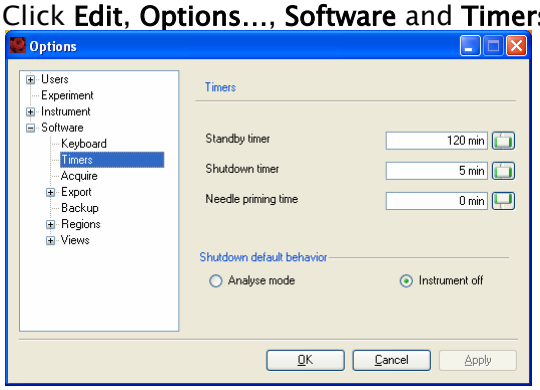


Figure 7.1 Timers window

2) Click on the **Shutdown time** field. Modify the time (minutes) using the slider bar or keyboard.

3) Click **Apply** and **OK**.

> **Note:** See section 6.11 for an overview of the MACSQuantify Software **Options** window.

8 MACSQuant Live support

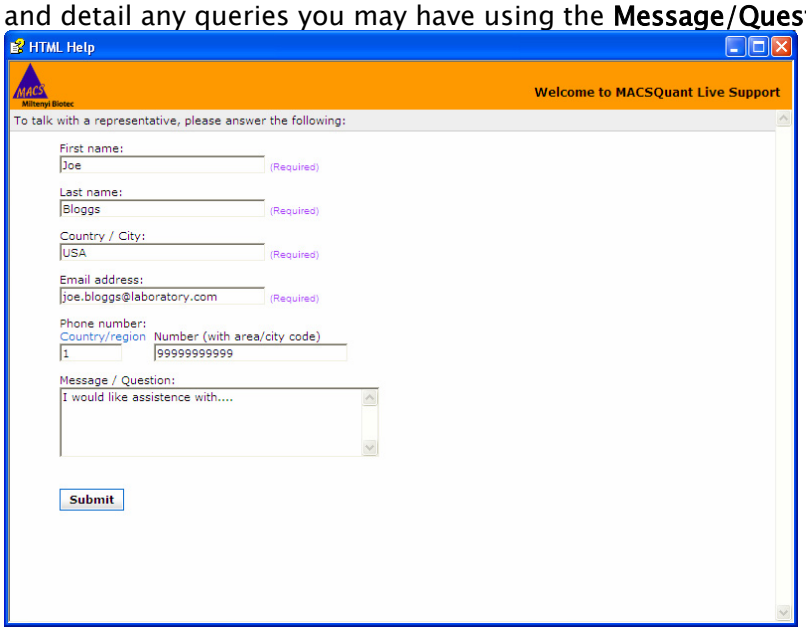
MACSQuant Live support is a real-time diagnostic service provided by Miltenyi Biotec technical support. Highly trained MACSQuant Experts can be reached in real-time to assist with any queries you may have.

Note: As an option is it possible to use a web-cam in communication with MACSQuant technical support. If no web cam is provided, please contact your nearest MACSQuant Specialist.

Note: The MACSQuant Analyzer must have network access to the internet for live support. Contact your local IT administrator if this is not the case.

To receive remote assistance:

1) Select the Tools menu.
2) Click **MACSQuant live support...**.
   A popup HTML field will appear. Complete the fields with your information and detail any queries you may have using the **Message/Question** box.



3) Click **Submit**.
4) Live support will commence.

9 Maintenance

9.1 General maintenance

The following section describes procedures required for maintenance of the instrument.

9.1.1 Pump maintenance

The syringe pump requires periodic maintenance. It is recommended to clean the dilutor housing and the syringe plunger every three months. Removing salt deposits as they appear can prevent leakage of the tubing system.

The pump seals or the syringe should be replaced once a year. Depending on the level of use and general instrument maintenance, however, these parts might need to be exchanged more frequently.

To exchange the pump syringe:

1) Make sure the fluidics system is shutdown. Switch OFF the power and unplug the MACSQuant Analyzer from the power outlet. Open the front access cover; the syringe pump plunger should be at its top position.

2) Unscrew the bottle closures (blue, green, black), and hold the bottle closures below the height of the dilutor-valve; gravity will draw any remaining fluids out of the tubing i.e. all fluids inside the tubing should be empty.

3) Remove the screws that attach the pump syringe safety guard and then remove the safety guard covering itself by lifting it.

4) Loosen the plunger lock screw by turning it counter-clockwise approximately three full turns.

5) Lower the plunger lock assembly by firmly pressing the plunger lock screw down.

6) Unscrew the syringe from the dilutor housing by turning it clockwise.

7) To install the newly cleaned syringe, carefully insert the syringe into the dilutor housing and screw counter-clockwise until resistance can be felt.

> **Note:** To clean the syringe, carefully remove the plunger from the syringe. Remove salt crusts with distilled or deionized water. Use distilled or deionized water to wet the plunger and carefully push the plunger back into the syringe. Dry the plunger lock screw before proceeding with installation of the syringe.

8) Push the plunger holder assembly up to the syringe plunger and tighten the plunger lock screw.

9) Turn on the MQ.

10) Prime the MACSQuant Analyzer.

> **Note:** If symptoms of wear such as leakage persist, contact the Technical Service.

9.1.2 Exchanging fluid containers

When one or more containers need replacing, follow the instructions below to ensure a safe exchange. Only exchange one container at a time and note the corresponding color coding of the container to be exchanged.

1) Unscrew the bottle closure counter-clockwise and remove. Close the container with a suitable (blue) cap and then remove the container from the fluid container holder. Do not disconnect the color-coded tubing from the bottle closure.

2) Place the new, sealed container in its appropriate position. Do not open a new fluid container until it is in position within the fluid container holder.

3) Unscrew and remove the (blue) cap; fasten the bottle closure securely.

4) Remove the bottle closure while the container is still in the fluid container holder.

5) Close the container with a cap and remove the bottle from the orange basket.

6) Exchange the container with a bottle containing approximately 100ml of disinfectant, e.g., 10% bleach.

7) Attach the bottle closure.

Note: Be extremely cautious with the waste fluids and dispose of in accordance with institutional or governmental regulations.

9.1.3 Exchanging the MACSQuant™ Column

The MACSQuant™ Column should be exchanged every 3 months. Exchange the column as outlined below.

Warning: Latex gloves and protective eyewear should be worn at all times to protect against potential biohazard exposure.

1) Open the front door and note the position of the tubing and MACSQuant™ Column in the MACS® Cell Enrichment unit (magnet).

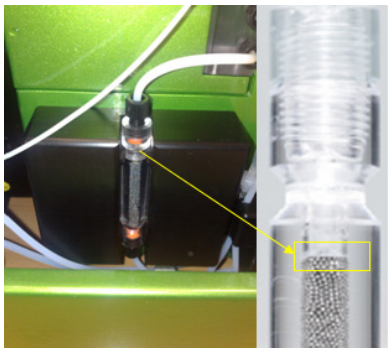


Figure 9.1 Position of the MACSQuant Column in the column holder

Note: The top end of the MACSQuant Column has a white filter (outlined in the red box). The column **must** only be inserted with the white filter at the top.

2) Using both hands, hold the top and bottom of the column and pull gently but firmly to remove it from its slot.

3) Hold the column in one hand and gently unscrew the upper column connector counter-clockwise. Tilt the column downwards to empty any fluid. Then,

unscrew the bottom column connector.

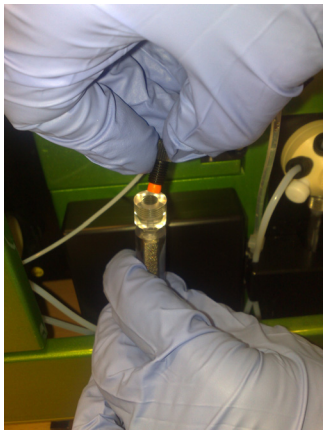


Figure 9.2The top connector is removed from the old column.

4) Insert the bottom end (no white filter) of the new MACSQuant™ Column into the bottom column connector and gently screw in the column by turning it clockwise until you feel resistance. Point the column towards the top of the device and screw in the top column connector.

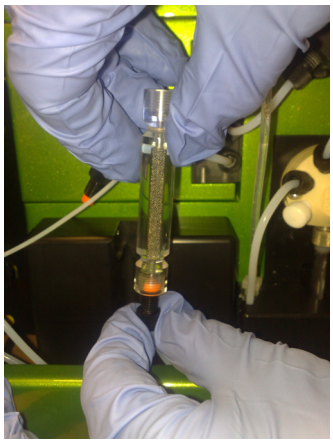


Figure 9.3 The bottom column connector is attached to the new column before attachment of the top connector

5) Align the column so that the top column connector sits on the guide of the magnet cover. Press the column into the slot until you feel the guides click. Verify that the column is placed in the center of the magnet cover.

6) Close the front door.

9.1.4 Exchanging fuses

If the instrument fails to start when switching it on or if operation suddenly stops and the screen is dark, an exchange of the fuses might be required. Follow steps 1 to 3

below to safely exchange the fuses.

1) Turn the instrument OFF.

2) Unplug the main power cord from the power outlet as well as from the instrument. The fuse holder is located below the main power connector on the rear panel of the instrument.

3) Pull out the fuse holder from the housing and replace with the appropriate fuses. Replace the fuse as instructed by the Miltenyi Biotec technical support, return the fuse holder to the housing and plug in the main power cord.

9.1.5 Exchanging the hydrophobic air filters

Hydrophobic air filters are attached to the bottle closures to vent the liquid bottles. To avoid clogging of the filters and to prevent contamination of the liquids, the air filters should be exchanged if they come into contact with liquid. They also should be exchanged once a year to avoid clogging through the deposition of dust.

9.1.6 Exchanging the pre-filter

The sheath particle filter provides a physical barrier to prevent debris larger than 70 µm in size from entering the fluidics system from the fluid containers. The filter can be easily exchanged when blocked, or at regular intervals to prevent blockages.

1) Shut down the fluidics system and remove the power cable from its socket.

2) Place a dish underneath the sheath filter to collect liquid.

3) Remove the filter unit from its holder and disconnect the tubing.

4) Connect a new filter to the tubing and reattach the filter to its holder.

9.1.7 Cleaning the washing station

1) Remove single tube holder if needed.

2) Open the front cover to the right side.

3) Open the cover of the washing station to the left side.

> **Note:** Potentially contaminated liquid may spill out of the orifice of the washing station and the tubing. Therefore, wear protective gloves, protective clothing, and safety glasses to avoid contact with skin and eyes.

4) Clean the washing station by wiping it with tissue and an appropriate disinfectant, e.g. 70% ethanol, isopropyl alcohol, or 10% bleach. Finally, rinse with distilled water.

9.1.8 Cleaning the uptake port

1) The uptake needle of the robotic arm should be cleaned regularly in order to prevent contamination or blockages.

   Turn OFF the instrument.

2) Wipe the needle with tissue soaked with 70% ethanol, isopropyl alcohol, or alcohol swabs followed by distilled or deionized water. Move the needle holder up and down to get access to the entire surface of the needle.

9.1.9 Decontaminating the MACSQuant® Analyzer

If the instrument has been used to process biohazardous samples, it is recommended to decontaminate the fluidics system by running the standard shutdown sequence. In case of spillage, it is recommended to use the appropriate disinfectant for the potential pathogen, e.g. MACS Bleach solution. Use tissues or swabs to decontaminate surfaces. The uptake needle of the robotic arm and the surface of the instrument also can be decontaminated upon contact with biohazardous samples.

Note:Dispose of tissues and swabs appropriately. It is recommended to wear protective gloves, protective clothing, and safety glasses to prevent contact with skin and eyes.

10 Troubleshooting

The instrument automatically analyzes the functionality of several hardware components during the instrument initialization procedure. An error message will appear if action is required by the user. Please see chapter 9, Maintenance, for help with dealing with certain error messages, otherwise contact the Technical Support.

10.1 Problems not indicated by error messages

This section addresses problems that are not indicated by a warning or error message that might occur during measurement. Cell separation, or rinsing programs. Identify the problem and refer to the appropriate section.

10.1.1 Column leakage

1) If a freshly installed MACSQuant® Analyzer Column shows signs of leakage, check if the column is installed properly. The column should be inserted correctly into the column connector and fastened to the point of resistance. If this is not the case, loosen the column connector, insert the column correctly, and tighten the connector.

2) If the column leakage persists, unscrew the column and check if the connectors of the columns are damaged. If this is the case, exchange the leaking column with a new MACSQuant® Analyzer Column.

3) If the problem still persists, contact the Technical Service.

> **Note:** Check that the column is not broken or cracked. In this event replace the column. See section 9.1.3 for more details.

10.1.2 Pump syringe leakage

1) Shut down the instrument.

2) Remove the pump syringe as described in section 9.1.3.

3) Carefully remove the plunger from the syringe. Wet a tissue with distilled water and clean both the plunger and the syringe. Remove all obvious salt crusts. Use distilled or deionized water to wet the plunger and carefully push the plunger back into the syringe.

4) Install the pump syringe as described in section 9.1.1. Make sure that the syringe is installed correctly into the syringe housing.

5) If the leakage persists, order and install either a new pump syringe (Order no. 130-090-339) or a new pump seal (130-022-101).

Note: Depending on the level of use, the pump syringe should be cleaned every 1-3 months.

Correct overnight and long-term storage assures that no salt deposits accumulate in the pump syringe. Salt deposits may cause wear of the pump seal and thus may lead to leakage.

The pump syringe should not run dry at any time. This can damage the pump seal and thereby may lead to leakage of the pump syringe.

10.1.3 Washing station overflow

Please contact the Technical Service.

10.1.4 MACS® MiniSampler does not move properly

Check whether the MiniSampler guide is inserted properly into the corresponding slot on the MACSQuant® Analyzer and ensure that the cable connecting the MiniSampler to the back of the instrument is not obstructed in any way.

10.1.5 Air bubbles during measurement or no events are acquired

Check for leakage within the fluidics and tubing system and perform a rinse program. Also, ensure that the pre-filter is devoid of air. Ensure that the needle calibration was performed correctly. Furthermore, check that the tubes connected to the bottle closures are tightly sealed. If the problem persists, contact the Technical Service.

10.1.6 Excessive debris is present in acquisition

Check all fluidics to ensure that all tubes are connected properly. Ensure that the pre-filter is bled of air.

Additionally, you can rinse or clean the system. Push the rinse button “teardrop”. Before this twice. If this does not work, try to clean the system by

10.1.7 Touchscreen remains dark

1) Check if the power cord is plugged in correctly and the power supply is switched on.

2) If the device is powered-up and the touchscreen remains dark, switch-off the device, wait 5 seconds, and switch on again. If the MACSQuant Analyzer still does not initialize, go to step 2.

3) Replace the fuses. Spare fuses are included in the MACSQuant Analyzer Starting Kit this is not true. If the problem persists, contact the Technical Service.

11 Hardware monitor

Using the Hardware Monitor the current hardware status of the MACSQuant Analyzer can be assessed at any time. The hardware pages can provide additional information on the status of the instrument in the case of error messages appearing on the screen. Never run the instrument if any errors are indicated within the hardware monitor.

11.1 Hardware Monitor window

The Hardware Monitor window can be accessed under the View menu. The window contains five tabs, each displaying the hardware components involved in the respective processes.

11.1.1 Fluidics components

The fluid containers will be illuminated in red when empty or the waste bottle is full. The waste, air, and sheath pump symbols are illuminated in green when they are active. Active separator valves (**Valves 1-3**) are indicated in green. Defective rotary valves are indicated in red. AS the system operates, the valves are displaced in the live status (o-open, c-closed)

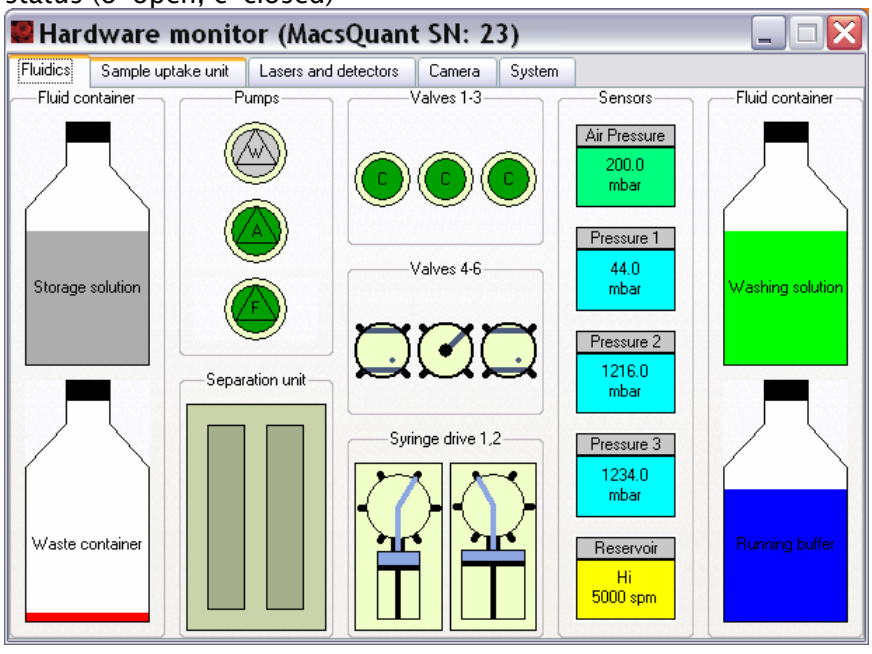


Figure 11.1 Real-time hardware monitor of the fluid components

Name	Further information
Fluid container monitor	Displays buffer/solution levels in real time
Pump monitor	Displays the status of the waste (W), air (A), and fill (F) pumps
Column monitor	Displays the status of the MACSQuant Column and MACS® Cell Enrichment Unit
Magnet valves monitor	Displays the position of the valves for the MACSQuant Analyzer Column: C = closed, O = open, green = in use
Rotary valve monitor	Displays the position of the general fluidics system valves
Dilutor monitor	Diplays the position of the dilutor
Sensor monitor	Displays the general system pressure and fluid reservoir levels.

Note: Air pressure: 200 mbar when the operating state is active.

Note: Laser temperature: Laser 488nm=37 °C; Laser 635 nm=25 °C; Laser 405 nm=25 °C

Note: Base plate temperature for 488 nm laser: 10-45

Note: Bench temperature=33 °C.

Note: Fan speed: This will fluctuate automatically depending on the ambient room temperature and internal temperature of the MACSQuant Analyzer.

Note: If errors are reported please refer to section 10 (troubleshooting) or Miltenyi Biotec technical support.

11.1.2 Sample uptake

The Sample uptake tab indicates the status of the robotic arm as well as whether the MACS® Mini Sampler is connected or not. The relative position of the needle arm is listed in the lower left box and if the single tube rack is connected.

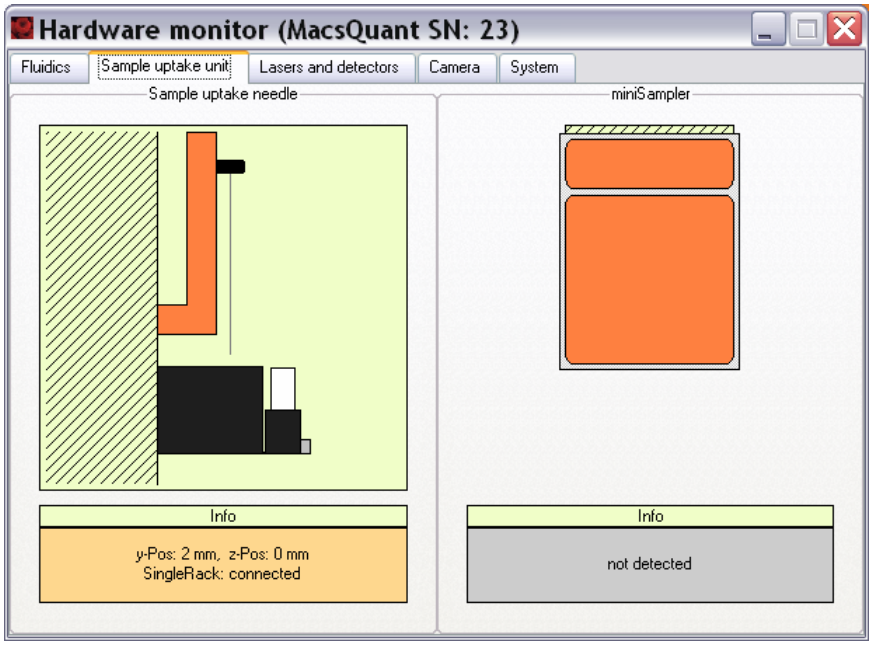


Figure 11.2 Real-time hardware monitor of the sample uptake arm

Name	Further information
Sample uptake needle schematic	Indicates the coordinates of the needle arm.
MACS MiniSampler monitor	The MiniSampler is not connected.

Liquid uptake rates

Measuring mode	Sheath pressure (mbar)	Buffer consumption for rinsing (µL)	Sheath buffer consumption during rinse cycle (µL)	Total volume (µL)	Total time (sec)
Fast	200	4166.6	750	4916.6	12
Standard	200	4875	1700	6575	25
Extended	200	5250	5800	11050	72

Sheath consumption for a 100 µL measurement (without rinsing volume)

Measuring mode	Sheath pressure (mbar) and flow rate (µL/min)	Measurement volume (µL)	Sheath buffer consumption during measurement cycle i.e. without rinse cycle (µL)	Total measurement time (min)
Standard	200 Low = 25 µl/min	100	18000	4
Standard	250 Med = 50 µl/min	100	13000	2
Standard	300 High = 100 µl/min	100	8600	1

11.1.3 Optical bench

Within the lasers and detectors tab it is possible to monitor the status of each laser fluorescence channel and detector. The temperature, fan speed PMT voltage and

annotated path of each laser is shown. Fluorescence channels that are active are highlighted. A status overview of the optical bench is schematically represented.

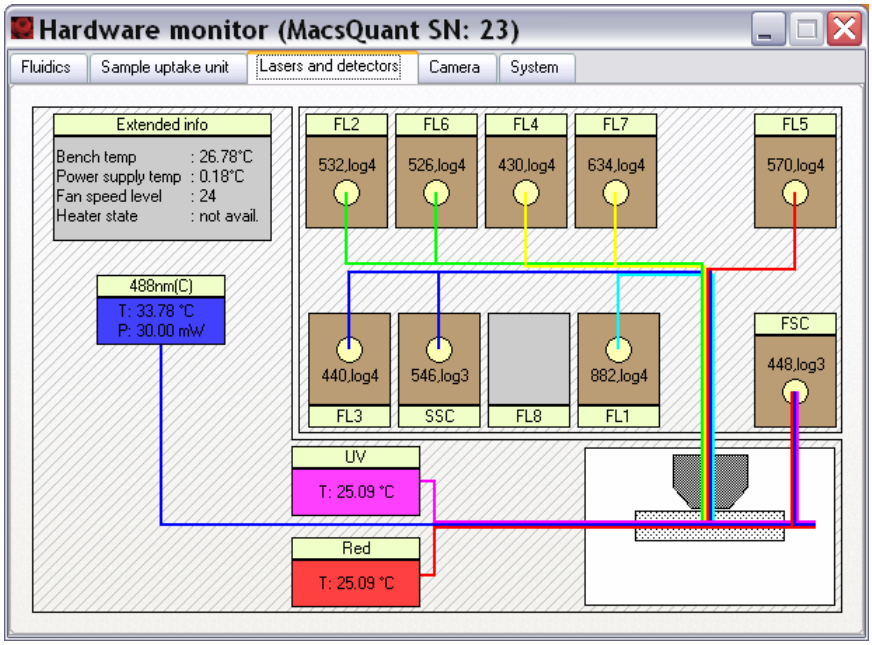


Figure 11.3 Real-time hardware monitor of the fluid components

12 Technical data and specifications

12.1 Labeled diagrams of the MACSQuant® Analyzer

The key accessible components of the MACSQuant Analyzer are schematically shown in figures 1 to 5, and can be roughly grouped into six categories comprising:

- the integrated computer and touchscreen
- the robotic arm and sample uptake port
- the access cover to tubing system (the fluidic system, including tubes and buffer bottles)
- the fluid containers and their holders
- the MACS MiniSampler and tube racks
- the plugs, connectors, and guides

Integrated computer for control of cell processing

All interactions with the computer may be performed using the TFT color touchscreen or via an externally connected keyboard and mouse. The computer is integrated within the MACSQuant Analyzer and any software updates can be easily performed using the DVD-RW drive, which is located at the top of the touchscreen monitor (Figure 12.1). The computer operating system is Microsoft® Windows® XP embedded and the all cell analysis and pre-enrichment programs are controlled by the MACSQuantify™ Software.

Automated arm with sample uptake port

The robotic arm (Figure 12.1) is a computer-controlled component of the MACSQuant Analyzer fluidics system. It consists of two motors that drive the arm in y and z directions and a needle for sample uptake. The needle and uptake port are automatically washed in the MACSQuant Analyzer washing station during and after cell processing to prevent cross-contamination between samples.

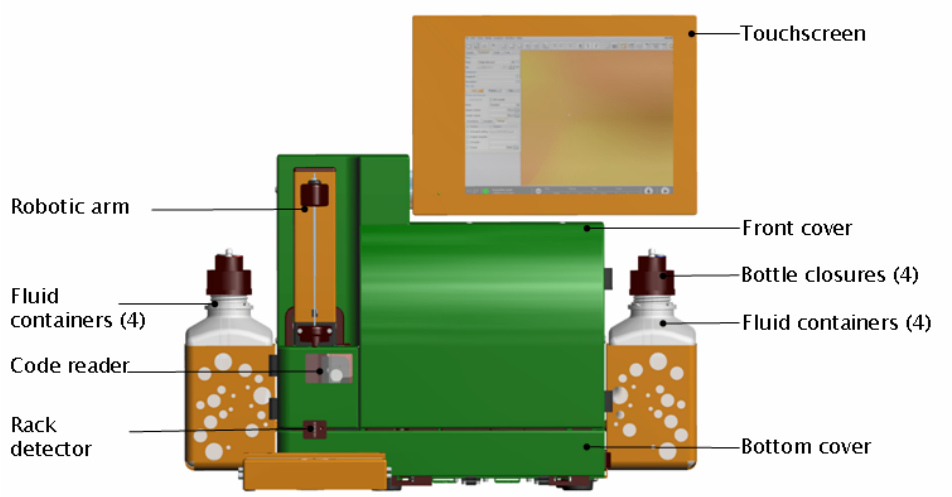


Figure 12.1 Front view of MACSQuant Analyzer

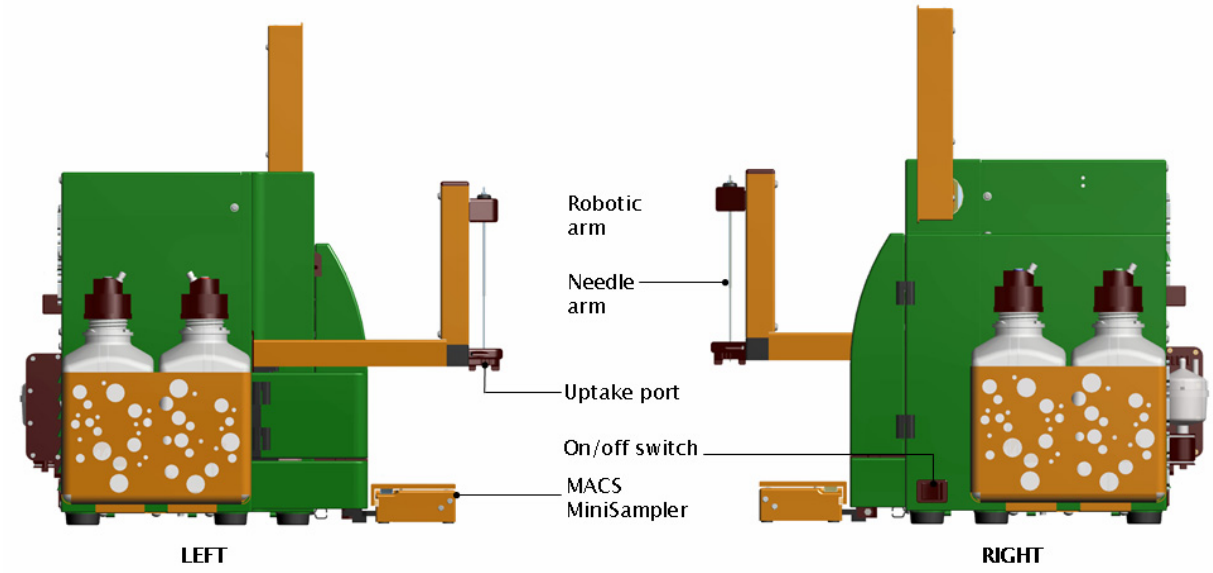


Figure 12.2 Left and Right views of the MACSQuant Analyzer

Access covers

The hinged front cover (Figure 12.1) can be opened to the right to allow access to the components of the fluidic system, including the syringe pumps, the MACSQuant Analyzer Enrichment Unit and MACSQuant™ Column (or substitute column) and the upper valves (Figure 12.3). The hinged washing station cover is opened to the left and gives access to the washing station, barcode readers, the peristaltic pump, and the tubing of the washing station. Both hinged covers can be removed by opening the door and lifting the covers vertically off of the guides. The bottom cover (Figure 12.1) gives access to the lower valves and can be removed by pulling gently toward you.

Fluidics system

The MACSQuant Analyzer fluidics system supplies the instrument with all buffers and solutions required during and after operation. The fluidics system (Figure 12.3) comprises of the tubing (with color-coded markers to assist correct connection to the respective fluid container) as well as their guides, three solenoid valves, three valves, a

flow cell, four pressure sensors, two syringe pumps and the MACSQuant Column (Figure 12.3).

MACSQuant Column

The MACSQuant Analyzer Column (Figure 12.3) is an optional component that is designed for the enrichment of cells using MACS® Technology before fluorescence cell analysis. Up to 5×10^7 cells from up to 5ml can be enriched in one isolation using the column; the column should be replaced every three months. For further details refer to the respective product data sheet.

Fluid containers and fluid container holders

Two orange baskets (Figure 12.1) holding two fluid containers each are located on either side of the instrument. The containers supply the MACSQuant Analyzer with running buffer for use during cell analysis, a washing solution for rinsing the instrument and a storage solution for overnight or long-term storage. A fourth bottle is used to collect all waste produced during operation of the instrument. Fluid levels within each container are constantly monitored by sensors contained within the bottle closures and a warning message appears on screen when action is required by the user.

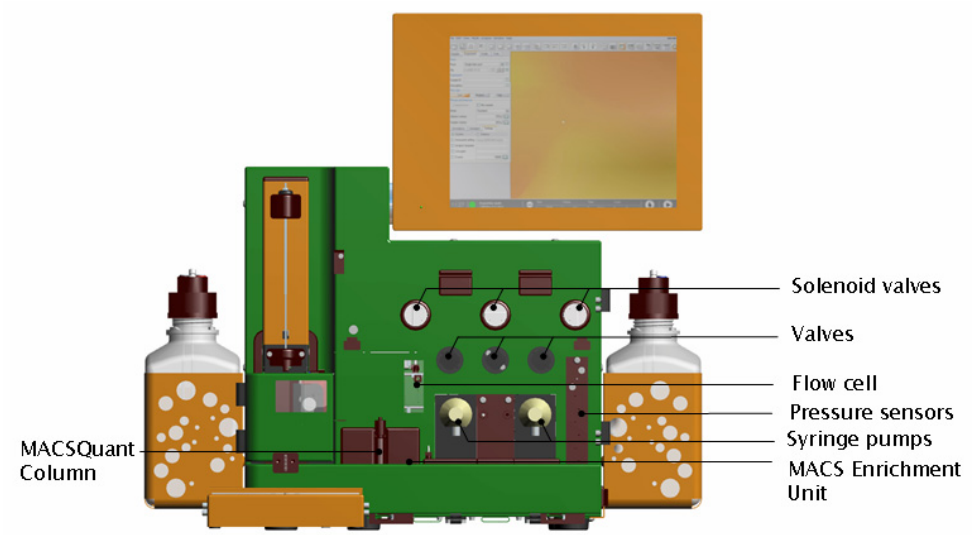


Figure 12.3 Front of MACSQuant Analyzer with access cover removed

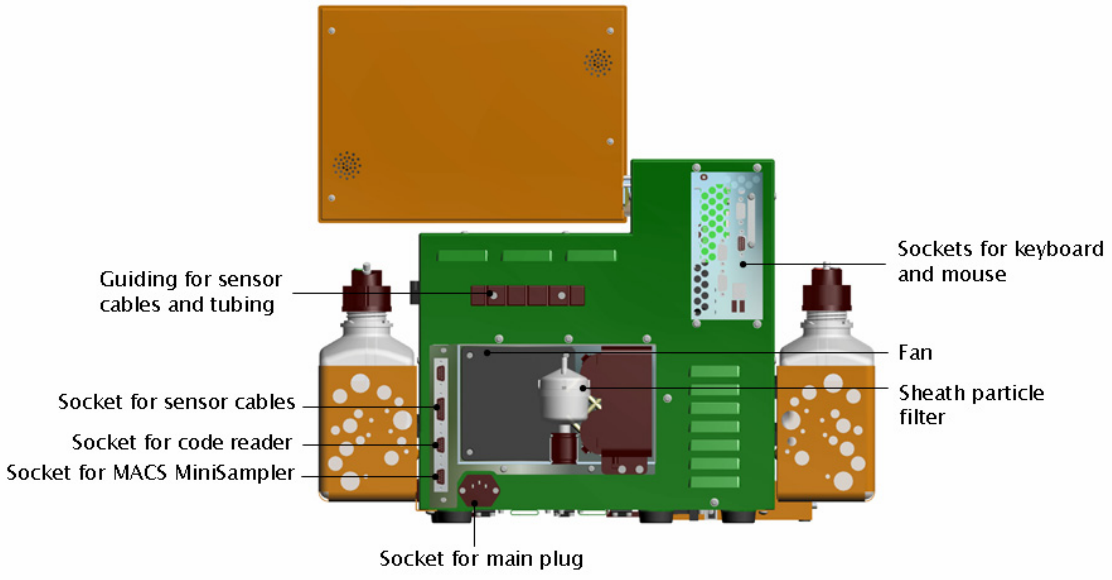


Figure 12.4 Rear view of the MACSQuant Analyzer

Air filter

The fluid particle filter provides a physical barrier to prevent debris larger than 70 µm in size from entering the fluidics system from the system buffer/cleaning solution containers. The fluid particle filter can easily be exchanged when blocked or at regular intervals to prevent blockages.

Plugs, connections, and guides

Sockets for the main power supply, the fluid sensor cables and the MACS MiniSampler are located at the rear of the instrument (Figure 12.4). Extra sockets have been provided for the case of further instrument development. The main power switch is located at the right-hand side of the instrument (Figure 12.2). Several guides at the rear and sides of the instrument ease the safe connection of tubing and sensor cables. Two USB ports, a VGA output port, an Ethernet port, and two serial ports are also included. The serial ports are used for attachment of the MACS MiniSampler and barcode reader.

MACSQuant Analyzer Fluid Containers

The MACSQuant Analyzer has two orange baskets on each side of the instrument that holds the three fluid containers required for operation of the MACSQuant Analyzer, which are MACSQuant Running, Washing and Storage solutions. A fourth in the orange buckets is reserved for the waste container. The bottle closures, the fluid sensor cables, and the tubing connectors are all color-coded and indicated by individual symbols for the specific position of each container on the MACSQuant Analyzer (see section 3.2.1 for more details).

Single tube holder

The single-tube holder can be used for the analysis of individual samples. Each measurement can be started using the orange button provided on the tube holder.

MACS MiniSampler, Chill Racks and MACS Reagent Rack

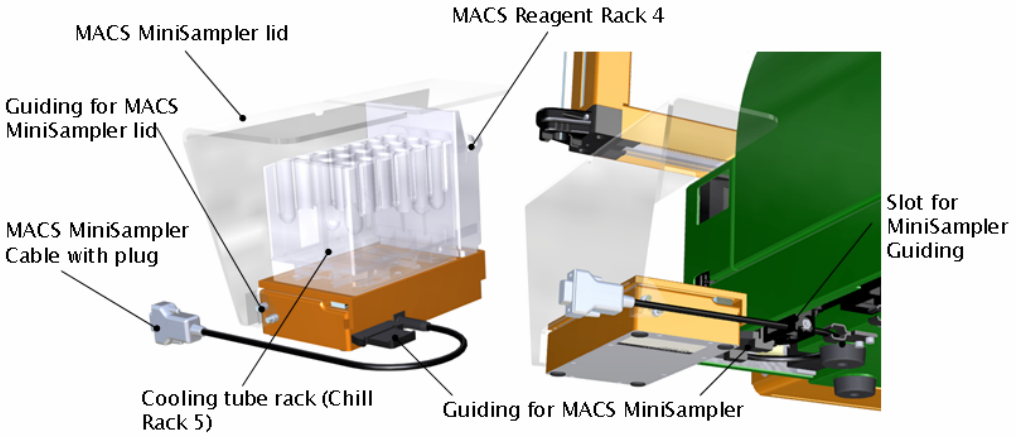


Figure 12.5 Rear view of MACS MiniSampler with MACS Reagent Rack and Chill Rack 5

The MACS® MiniSampler (Figure 12.5), an optional attachment, is designed for automated, multiple sample processing by the MACSQuant Analyzer. The MACS MiniSampler can be loaded with MACS Chill Racks that hold cell samples and cell separation fractions. The upper plate of the Mini Sampler moves horizontally (*x*-plane of direction) and aligns the tube openings with the port of the automated arm. The guide of the MiniSampler is directly attached to the corresponding slot located below the washing station. The MiniSampler is automatically detected upon attaching the MiniSampler sensor cable to the corresponding socket at the rear of the instrument. The type of tube rack carried by the MiniSampler can be automatically recognized by the rack detector using the rack detection barcode reader (Figure 12.6) or the user can specify which rack will be used. During operation, the tube rack should be covered with the Mini Sampler lid that is connected to the lid guide. The MiniSampler can be disconnected from the MACSQuant Analyzer by gently lifting the MiniSampler in an upward direction followed by carefully pulling the device away from the MiniSampler slot located on the MACSQuant Analyzer.

2D code reader

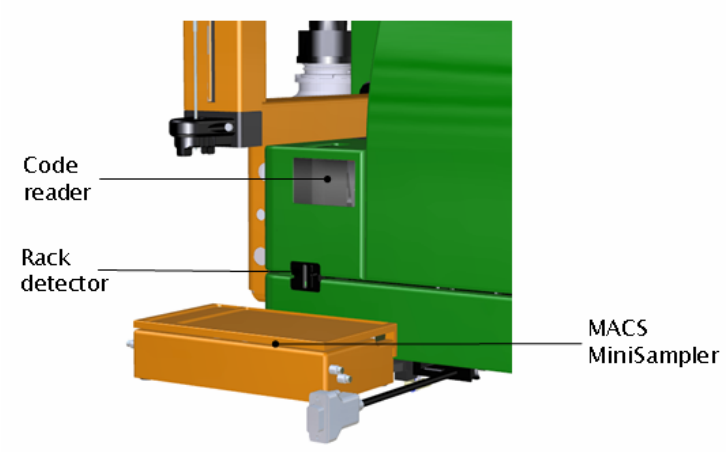


Figure 12.6 Expanded view of the MACSQuant Analyzer 2D code reader ("barcode reader")

The MACSQuant Analyzer is equipped with a 2D code reader that uses lasers and powerful light-emitting diodes (LEDs) for illuminating the reading area. The 2D code

reader light is classified as a Class 1 laser product per standard IEC 60825-1:1993 + A1:19976 + A2: 2001 (maximum output 116 µW; wavelengths 655 nm, pulse duration 1 ms). Please refer also to section 1.3.4 (Important Information) of the MACSQuant Analyzer Manual for associated warning and precautionary information.

The technical specifications of the MACSQuant Analyzer and the peripheral devices are as follows:

12.2 Technical data and specifications of the MACSQuant® Analyzer

The MACSQuant® Analyzer is labeled as a protection class I device and must be plugged into a grounded power outlet, see section 1.2 (Warnings and precautions). The main power supply cord and plug of the instrument shall comply with following specifications (USA and Canada only): UL listed and KAM cord, minimum type SJ, minimum 18 AWG, 3 conductors. Rated for a minimum temperature of 60 °C. Provided with grounding-type (NEMA 5-15P) attachment plug, rated 125 VAC, 10 A. Opposite end terminates in IEC 320 style connector, rated 125 VAC, 10 A.

Parameter	Specification
Color	Green/orange
Footprint	605 mm × 400 mm (w × d)
Footprint with MiniSampler	605 mm × 500 mm (w × d)
Height	392.5-520 mm (adjustable touchscreen)
Weight	50 kg

Table 12.1 Dimensions of the MACSQuant® Analyzer

Parameter	Specification		Parameter	Specification
Model	MACSQuant® Analyzer		CAN Bus + DC-Output (labeled "External CAN")	Pins 1, 4, 8: NC Pin 2: CAN-L Pins 3, 6: GND Pins 5, 9: 24 VDC / 2A Pin 7: CAN-H
Input voltage	100-240 VAC, ~50/60 HZ		AC Output (labeled "Bottle Sensor")	Pins 1, 2, 3, 4, 5: 5 VAC / 10 kΩ Pins 6, 7, 8, 14, 15: GND Pins 9, 10, 11, 12, 13: Input
Power consumption	350 W		CAN Bus (labeled "CAN1" or "CAN2")	Pins 1, 4, 5, 8, 9: NC Pin 2: CAN-L Pins 3, 6: GND Pin 7: CAN-H

Parameter	Specification		Parameter	Specification
Model	MACSQuant® Analyzer		CAN Bus + DC-Output (labeled "External CAN")	Pins 1, 4, 8: NC Pin 2: CAN-L Pins 3, 6: GND Pins 5, 9: 24 VDC / 2A Pin 7: CAN-H
Fuses	2 × 5AT, 250V		USB port (labeled "USB")	Pin 1: USB1_+5V Pin 2: USB1- Pin 3: USB1+ Pin 4: USB1_GND Pin 5: USB2_+5V Pin 6: USB2- Pin 7: USB2+ Pin 8: USB2_GND
RS232 Interface (labeled "COM 1")	Pin 1: DCD Pin 2: RxD Pin 3: TxD Pin 4: DTR Pin 5: GND Pin 6: DSR Pin 7: RTS Pin 8: CTS Pin 9: RI		Ethernet port (labeled "Ethernet")	Pins 4, 5, 7, 8: NC Pin 1: TXD+ Pin 2: TXD- Pin 3: RXD+ Pin 6: RXD-
RS232 Interface (labeled "RS232/AUX") Not in use	Pins 1, 4, 6, 7, 8, 9: NC Pin 2: RXD Pin 3: TXD Pin 5: GND		VGA Interface (labeled "VGA")	Pin 1: RED Pin 2: GREEN Pin 3: BLUE Pin 5: GND Pin 6: GND Pin 7: GND Pin 8: GND Pin 10: GND Pin 13: HSYNC Pin 14: VSYNC
RS232 Interface + DC-Output (labeled "RS232/BCR")	Pins 4, 6: NC Pin 1: Input Pin 2: RXD Pin 3: TXD Pin 5: GND Pins 7, 8: Shorted Pin 9: 5 VDC / 0.5 A			

Table 12.2 Power and interface/port technical specifications of the MACSQuant® Analyzer

Conditions of operation

Conditions of operation: 20–25 °C with 0–85% humidity at a maximum altitude of 2,000 m. Supply voltage fluctuations up to ±10% of the nominal voltage. Transient over voltages (voltage in excess of the normal operating voltage) present on the mains supply: Category II. The instrument is suitable for rated pollution degree 2. The MACSQuant Analyzer is not specified for use in a cold room.

The MACSQuant Analyzer has been investigated by Underwriter Laboratories in accordance with the standarads UL 61010-1, CAN/CSA-C22.2 No. 61010-1, and IEC 61010-2-081 and meets the intent of the directive 2004/108/EC (electromagnetic compatibility) and 2006/95/EC (low voltage equipment). Compliance was demonstrated by conformance to the following harmonized European Standards which have been listed in the Official Journal of the European Communities:

EMC:	EN 61326-1
	EN 61000-3-2
	EN 61000-3-3
Low voltage equipment:	EN 60825-1
	EN 61010-1
	EN 61010-2-081

Compliance was demonstrated by conformance to the following Codes of Federal Regulations:

47 CFR §15, class B	CDRH 21 CFR 1040.10 and 1040.11

12.3 Technical data and specifications of the MACS® MiniSampler

Model	MACS® MiniSampler
Size without lid	182 mm × 148 mm × 47 mm
Size with lid	280 mm × 153 mm × 172 mm
Weight	1.5 kg
Input voltage	24 VDC
Current	0.8 A
Sub D9 interface with shielding	Pin 1: NC Pin 2: CAN- Pin 3: GND Pin 4: NC

Pin 5: +24 V Pin 6: GND in 7: CAN+ Pin 8: NC Pin 9: +24 V

Table 12.3 Technical data and pin assignment for MACS MiniSampler

Note: The MACS MiniSampler is labeled as a protection class III device and must only be plugged into the connector labeled with “external CAN” on the MACSQuant Analyzer. For further details see section 1.2, Warnings and precautions.

Note: The MiniSampler unit is an optional extra. If the MiniSampler is not ordered a dongle will be shipped with the MACSQuant Analyzer. It is essential to attach the dongle to the external MiniSampler CAN socket located at the back of the MACSQuant Analyzer.

The MACS MiniSampler is labeled as a protection class III device and must only be plugged into the connector labelled with “External CAN” of the MACSQuant Analyzer, see chapter warnings and precautions.

The MACS MiniSampler is designed for operation with three different tube racks and a reagent rack.

Rack type	Slots
Chill 5	24 × 5 mL
Chill 15	15 × 15 mL 5 × 5 mL
Chill 50	6 × 50 mL 3 × 15 mL 3 × 5 mL
Chill 96 rack/ 96 rack	96-well microtiter plate

Table 12.4 MACS Cooling Tube Racks: Chill Racks 5, 15 and 50

Rack type	Slots
MACS Reagent Rack 4	4 × MACS Reagent vials

Table 12.5 MACS Reagent Rack 4

Conditions of operation: 15–30 °C with 0–85% humidity at a maximum altitude of 2000m.

The MACS MiniSampler is not specified for use in the cold room.

The MACS MiniSampler has been investigated by Underwriters Laboratories in accordance with the standards UL 61010-1 and CAN/CSA -C22.2 No. 61010-1 and meets the intent of the directive 2004/108/EC (electromagnetic compatibility). Compliance was demonstrated by conformance to the following harmonized European Standards which have been listed in the Official Journal of the European Communities:

EMC: EN 61326-1

EN 61000-3-2

EN 61000-3-3

Compliance was demonstrated by conformance to the following FCC Rules of the Code of Federal Regulations:

47 CFR §15, class B

13 Technical service

Miltenyi Biotec offers a full range of customer technical support options for your MACSQuant Analyzer.

For support and technical questions, or if you think your MACSQuant Analyzer is malfunctioning, please contact your local Miltenyi Biotec representative or Miltenyi Biotec's technical support team:

Germany/Austria/Switzerland
Miltenyi Biotec GmbH
Friedrich-Ebert-Str. 68
51429 Bergisch Gladbach
Germany
Phone +49 2204 8306-830
Fax +49 2204 8306-89
macsTec@miltenyibiotec.de

USA/Canada
Miltenyi Biotec Inc.
12740 Earhart Avenue
Auburn, CA 95602, USA
Phone +1 530 888 8871
Fax +1 530 888 8925
Toll free 800 FOR MACS
Toll free +1 800 367 6227
macs@miltenyibiotec.com

Australia
Miltenyi Biotec Australia Pty. Ltd.
Unit 16A, 2 Eden Park Drive
North Ryde, NSW 2113
Australia
Phone +61 02 8877 7400
Fax +61 02 9889 5044
macs@miltenyibiotec.com.au

Benelux
Miltenyi Biotec B.V.
Postbus 85183
3508 AD Utrecht
Netherlands
Customer service, Belgium
Phone 0800 94016
Fax 0800 99626
macs@miltenyibiotec.nl

Customer service, Luxembourg
Phone 800 24971
Fax 0800 24984

Customer service, Netherlands
Phone 800 4020120
Fax 0800 4020100

China
Miltenyi Biotec Shanghai Office
Fareast International Plaza A, Rm. 2301
No. 319 Xianxia Rd.
Shanghai 200051, P.R.China
Phone +86 21 62351005
Fax +86 21 62350953
macs@miltenyibiotec.com.cn

Singapore
Miltenyi Biotec Asia Pacific Pte Ltd.
7, Temasek Boulevard #16-06
Suntec Tower One
Singapore 038987
Phone +65 6238 8183
Fax +65 6238 0302
macs@miltenyibiotec.com.sg

Japan
Miltenyi Biotec K.K.
Nittsu-Etai Building 5F
16-10 Fuyuki, Koto-ku,
Tokyo 135-0041, Japan
Phone +81 3 56 46 8910
Fax +81 3 5646 8911
macs@miltenyibiotec.jp

France
Miltenyi Biotec
10 rue Mercoeur
75011 Paris, France
Phone +33 1 56 98 16 16
Fax +33 1 56 98 16 17
macs@miltenyibiotec.fr

Italy
Miltenyi Biotec S.r.l.
Via Persicetana, 2/D
40012 Calderara di Reno (Bologna)
Phone +39 051 64 60 411
Fax +39 051 64 60 499
macs@miltenyibiotec.it

Spain
Miltenyi Biotec S.L.
C/ Luis Buñuel 2
Ciudad de la Imagen
28223 Pozuelo de Alarcón (Madrid)
Phone +34 91 512 1290
Fax +34 91 512 1291
macs@miltenyibiotec.es

United Kingdom
Miltenyi Biotec Ltd.
Almac House
Church Lane, Bisley
Surrey GU24 9DR
Phone +44 1483 799 800
Fax +44 1483 799 811
macs@miltenyibiotec.co.uk

14 Limited warranty

Except as stated in a specific warranty statement, which may accompany your MACSQuant Analyzer (the “Product”), or unless otherwise agreed in writing by an authorized representative of Miltenyi Biotec, Miltenyi Biotec’s warranty, if any, with respect to this Product is subject to the terms and conditions of sale (the “Terms”) of the company within the Miltenyi Biotec group which supplied the Product. The Terms may vary by country and region. Copies of these Terms are available on request or at www.miltenyibiotec.com.

Nothing in this document should be construed as constituting an additional warranty.

Miltenyi Biotec’s product warranty only covers Product issues caused by defects in material or workmanship encountered during ordinary use, as described in the user manual or other documentation provided by Miltenyi Biotec; it does not cover Product issues not arising out of defects in material or workmanship, including but not limited to Product issues resulting from: failure to follow installation, operating and/or maintenance instructions, or environmental conditions prescribed in, this user manual or other Product documentation; misuse; abuse; neglect; mishandling; unauthorized or improperly performed maintenance or repairs; accident; acts of God; limitations of technology; electrical current fluctuations; modification of or to any part of the Product; use of accessories, spare parts and/or consumables other than those recommended by Miltenyi Biotec; or normal wear and tear. Miltenyi Biotec’s product warranty does not cover products sold AS IS or WITH ALL FAULTS, or which had its serial number defaced, altered or removed, or any consumables, or parts identified as being supplied by a third party; those third-party accessories or parts may be covered by a separate warranty from their manufacturer.

Miltenyi Biotec must be informed immediately, if a claim is made under such warranty. If a material or manufacturing defect occurs within the warranty period, Miltenyi Biotec will take the appropriate steps to restore the full usability of your Product.

Limitation on damages:
Miltenyi Biotec shall not be liable for any incidental or consequential damages for breach of any express or implied warranty or condition on this Product.

Some states or jurisdictions do not allow the exclusion or limitation of incidental or consequential damages, so the above limitations or exclusions may not apply to you. This warranty statement gives you specific legal rights and you may have other rights, which may vary from county to country or jurisdiction to jurisdiction.

15 Glossary

Air filter	Hydrophobic 0.2 µm air filter attached to the bottle closure. Used to vent the bottle and simultaneously prevent contaminants from entering or escaping from the fluid bottle.
Air filter connector	Luer-to-thread connector for attaching the air filter to the threaded bottle closure vent.
APC	Allophycocyanin.
MACSQuant™ Column	Separation column specifically designed for the MACSQuant® Analyzer.
MACSQuant® Analyzer:	An automated flow cytometer that is also referred to as device or instrument.
MACSQuant™ Running Buffer	Sterile and ready-to-use buffer for flow cytometry, cell enrichment, and washing programs. The tubing connector is color-coded blue.
MACSQuant™ Storage Solution	Sterile and ready-to-use solution for overnight and longterm storage of the MACSQuant® Analyzer. The tubing connector is color-coded black.
MACSQuant™ Washing Solution	Sterile and ready-to-use solution for washing and special cleaning programs. The tubing connector is color-coded green.
Bottle closure	Vented screw-on closure with fluid uptake tubes. The bottle closures contain fluid sensors and are equipped with sensor cable connectors. Each bottle closure is color coded
Column connector	Luer-to-thread connector holding the MACSQuant® Analyzer Column.
Column substitute	Column devoid of any paramagnetic particles. This column substitute can be used when enrichments are not being performed or for long-term storage and shipment. The column substitute cannot be used for cell enrichment prior to analysis.
FITC	Fluorescein isothiocyanate.
Fluid container	1.5 L bottle that holds the system fluids for operational use of the MACSQuant® Analyzer. Fluid sensors monitor the fluid levels in the containers for Running Buffer, Washing Solution, Storage Solution, and waste via electrolyte conductivity.
Fluid sensors	These sensors measure electrolyte conductivity and is integrated into the closures of the Running Buffer, Washing Solution , Storage Solution and waste fluid containers.
Fluid sensor cable	Cable connecting the fluid sensor to the MACSQuant® Analyzer. The sensor cables are color-coded: blue for Running Buffer, green for Washing Solution, black for Storage Solution and red for waste.
Front panel:	The front panel opens sideways, giving access to the MACSQuant® Analyzer Column, pumps, valves, washing station, and tubings.
MACS® Technology:	Technology for immunomagnetic labeling and subsequent separation of cells or biomolecules in a high-gradient magnetic field.

MACS® MicroBeads	Superparamagnetic particles conjugated to antibodies for magnetic labeling of cells or biomolecules.
MACS Cell Enrichment Unit – not in order	Black cover surrounds the area enclosing the magnet. The magnet cover is located in the center of the fluidics system and has a slot for the insertion of the MACSQuant™ Column.
Negative fraction	Sample fraction containing the cells not magnetically labeled. During MACS® Separation, these cells are not retained on the column and pass directly through the column while the column is in the presence of the MACS® magnet.
PE	R-Phycoerythrin
Positive fraction	Sample fraction containing the cells labeled with MACS® MicroBeads after MACS Separation. These cells are retained on the column while the column is placed in the magnetic field. The cells are eluted from the column after the column has been removed from the magnet.
Cell enrichment	Process of cell enrichment is accomplished by the MACS Cell Enrichment Unit and the MACSQuant™ Column. Target cells labeled with MACS® MicroBeads are enriched using MACS Technology.
Syringe pumps	Computer-controlled, high precision syringe pumps with Teflon® seal plunger that drive fluids through the MACSQuant® Analyzer fluidics system.
Touchscreen	High resolution TFT color touchscreen located on top of the MACSQuant® Analyzer. The touchscreen is used to operate and monitor the instrument via on-screen menus.
Tubing connector	Threaded plastic connector with a square nut used to connect the tubings to the bottle closures, the column, the pump, or valves.
Tubing system	Permanent set of Teflon® tubing through which fluid circulates in the MACSQuant® Analyzer fluidics system
Chill Tube racks	Different acrylic tube racks are available with the instrument and are designed for optimal positioning of sample tubes. They contain a coolant allowing racks to be pre-cooled in the refrigerator for subsequent cooling of the cells during analysis.
Uptake needle	The automated arm carries a needle port designed for computer-controlled uptake of sample. This needle is calibrated and self-cleaned. It is designed to move in the y and z direction.
Waste container	Container for waste fluid. The closure is equipped with a fluid sensor. The closure, the fluid sensor cable, and the tubing connector are color-coded red.

Miltenyi Biotec

Miltenyi Biotec GmbH
Friedrich-Ebert-Straße 68
51429 Bergisch Gladbach
Germany
Phone +49 2204 8306-0
Fax +49 2204 85197
macs@miltenyibiotec.de

www.miltenyibiotec.com

Miltenyi Biotec Inc.
12740 Earhart Avenue
Auburn CA 95602, USA
Phone 800 FOR MACS,
+1 530 888 8871
Fax +1 530 888 8925
macs@miltenyibiotec.com

Miltenyi Biotec Pty. Ltd. (Australia)
Phone +61 02 8877 7400
macs@miltenyibiotec.com.au

Miltenyi Biotec B. V. (Benelux)
macs@miltenyibiotec.nl

Customer service, Netherlands
Phone 0800 4020120

Customer service, Belgium
Phone 0800 94016

Customer service, Luxembourg
Phone 800 24971

Miltenyi Biotec Shanghai Office
Phone +86 21 6235 1005
macs@miltenyibiotec.com.cn

Miltenyi Biotec SAS (France)
Phone +33 1 56 98 16 16
macs@miltenyibiotec.fr

Miltenyi Biotec S.r.l. (Italy)
Phone +39 051 646 0411
macs@miltenyibiotec.it

Miltenyi Biotec K.K. (Japan)
Phone +81 3 56 46 8910
macs@miltenyibiotec.jp

Miltenyi Biotec Asia Pacific Pte. Ltd. (Singapore)
Phone +65 6238 8183
macs@miltenyibiotec.com.sg

Miltenyi Biotec S.L. (Spain)
Phone +34 91 512 12 90
macs@miltenyibiotec.es

Miltenyi Biotec Ltd. (UK)
Phone +44 1483 799 800
macs@miltenyibiotec.co.uk